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DTU Environment
Department of Environmental Engineering

PhD Thesis
April 2012

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PREFACE

The work presented in this PhD thesis was carried out at the Technical University of Denmark's Department of Environmental Engineering under supervision of Mette Martina Broholm (primary supervisor) and Poul Løgstrup Bjerg (co-supervisor). The work was conducted from July 2008 to March 2012 as a part of the project Innovative REMediation and assessment TEChnologies for contaminated soil and groundwater (REMTEC). The primarily funding was provided by the Strategic Research Committee while the Capital Region of Denmark provided funding for parts of the field investigations. The PhD thesis comprises a synopsis of the work presented in two published papers, two manuscripts prepared for scientific journals and a technical note. In the synopsis of the thesis the papers are referred to by the names of the authors and the Roman numerals (e.g. Damgaard et al., III). The papers included in the thesis are:

I. Christiansen, C. M., Damgaard, I., Broholm, M., Kessler, T., Klint, K.E., Nilsson, B., and Bjerg, P.L., 2010. Comparison of Delivery Methods for Enhanced In Situ Remediation in Clay Till. Ground Water Monitoring & Remediation, 30(4): 107-122.

II. Christiansen, C. M., Damgaard, I., Broholm, M. M., Kessler, T., and Bjerg, P. L., 2012. Direct-Push Delivery of Dye Tracers for Direct Documentation of Solute Distribution in Clay Till. Journal of Environmental Engineering, 138(1): 27-38.

III. Damgaard, I., Bjerg, P. L., Bælum, J., Scheutz, C., Hunkeler, D., Jacobsen, C. S., Tuxen, N., and Broholm, M. M., 2011. Integrated characterization of chlorinated ethenes and ethanes degradation in clay till. Manuscript, Submitted.

IV. Damgaard, I., Bjerg, P. L., Jacobsen, C. S., Tsitonaki, A., Kerrn-Jespersen, H., and Broholm, M. M., 2011. Performance of full scale bioremediation in clay till using enhanced reductive dechlorination. Manuscript. Submitted.

V. Chambon J., Damgaard, I., Broholm, M., Hunkeler, D., Binning, B. and Bjerg, P. L. 2011 Isotope data for dechlorination in clay tills – Use of modeling, Technical note.

The main focus of my PhD was on investigating the development of degradation in the clay till matrix (Damgaard et al., III, IV and V). The bulk part of the work for scientific paper I and II was planned by Camilla Mayman Christiansen. I assisted in the planning and execution of field tests and laboratory work and authored the report for the funding agency.

The work has been presented at Danish and international conferences both orally and through poster presentations. Two reports have also been prepared for the Capital Region of Denmark.

The papers are not included in this www-version. The papers can be obtained from the Library at DTU Environment.

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SUMMARY (ENGLISH)

Chlorinated solvents are among the most frequently found contaminants in groundwater. In fractured media, chlorinated ethenes and ethanes are transported downwards through preferential pathways with subsequent diffusion into the sediment matrix. Due to slow back diffusion it can serve as a long term secondary source that can leach to the underlying aquifer. As some of the chlorinated solvents and their degradation products are toxic and carcinogenic, remediation technologies applicable in low permeability settings are needed.

Enhanced reductive dechlorination (ERD) has been proven efficient in high permeability aquifers and has also been applied at a number of low permeability clay till sites. This thesis presents the results of an investigation of chlorinated ethenes (and ethanes) degradation in clay till with the objective of obtaining knowledge of degradation processes in clay till and to evaluate ERD as remediation technology. The development of degradation in clay till was investigated at two sites: one where natural attenuation processes (transport, sorption, diffusion and degradation) had been on-going for four decades (Vadsbyvej) and another which had been undergoing ERD for four years (direct push delivery, Gl. Kongevej). Degradation of chlorinated ethenes (and ethanes) in the clay till matrix and in embedded high permeability features was investigated by high resolution sampling of intact cores combined with groundwater sampling. An integrated approach using chemical analysis, molecular microbial tools and compound specific isotope analysis (CSIA) was used. The results from the full scale investigation were compiled with another full scale ERD remediation in clay till (gravitational injection, Sortebrovej, Denmark).

The study of on-going natural degradation of chlorinated ethenes and ethanes in clay till (Vadsbyvej) revealed a very complex system where diffusion, biotic and abiotic degradation processes occurred simultaneously. High resolution sub sampling with combined use of chemical analysis, molecular microbial tools and CSIA was necessary to identify both biotic and abiotic degradation zones. Reductive dechlorination of TCE to cis-DCE had developed in entire clay till sections of up to 3 m, whereas sub-sections with partial degradation to VC and ethene were more sporadically distributed due to scarcity of *Dehalococcoides* with the functional gene *vcrA*. The study shows the potential for development of degradation throughout the entire clay matrix.

When ERD is applied in a low permeability settings one of the major constraints is to obtain the necessary contact between electron donor, bacteria and contaminants to achieve reasonable remediation timeframes. Two injection methods (hydraulic fracturing with gravitational injection and direct push delivery) were therefore tested in clay till by injection of amendment-comparable tracers to investigate the possibility to overcome diffusion limitations in the low permeability matrix. The study of hydraulic fracturing demonstrated that it was only possible to create a horizontal fracture in 3 m b.s., whereas it was not successful between 6-9.5 m b.s. at the test site. Closely spaced (10 and 25 cm) horizontal delivery of amendment-comparable tracers was achieved by direct push delivery using a GeoProbe® from 2.5 to 9.5 m b.s. Contrary to these results, fractures were not in all cases observed for every 25 cm (the injection interval) after injection of electron donor and bacteria by direct push delivery.

The primary propagation path for organic substrate and bacteria was natural sand stringers and sand lenses. However, by direct push delivery organic substrate was also spread in natural or induced fractures. After four years of ERD in clay till, reductive dechlorination of chlorinated ethenes had developed very heterogeneously in the clay till matrix after both gravitational injection (Sortebrovej) and direct push delivery (Gl. Kongevej). In some areas degradation was restricted to narrow zones around soft clay till, sand stringers and sand lenses, and in other sections degradation had developed through entire sections of the clay till matrix (up to 1.8 m at Gl. Kongevej). Only minor or no degradation developed in the untreated intervals. Reductive dechlorination in the clay till matrix at Gl. Kongevej was documented by enriched isotope fractionations of TCE and cis-DCE and the presence of *Dehalococcoides* with the *vcrA* gene in the clay till matrix. The degradation of chlorinated ethenes in the clay till matrix was not as advanced as in the high permeability features indicating that sediment analysis is needed to evaluate the performance of ERD in clay till.

The shortest remediation timeframes were found in areas where degradation had developed more extensively in the clay till matrix (approximately 20 years), whereas longer remediation timeframes were found when degradation was restricted to narrow reaction zones around sand stringers and sand lenses (up to 170 years). This illustrates the necessity of developing degradation in the entire clay till matrix to obtain reasonable timeframes of the remediation.

DANSK SAMMENFATNING

Chlorerede opløsningsmidler er blandt de oftest trufne forureningsstoffer i grundvandet. I et opsprækket medie som moræneler transporteres chlorerede opløsningsmidler initielt gennem preferentielle spredningsveje (sand slirer, sand linser og sprækker) med efterfølgende diffusion ind i lermatricen. På grund af langsom tilbage diffusion vil forureningen udgøre en langvarig kilde til forurening af underliggende grundvandsmagasiner. Eftersom flere af de chhlorerede ethener og ethaner er giftige og kræftfremkaldende, er der behov for oprensningsmetoder til lav permeable aflejringer.

Stimuleret reductiv dechlorering (ERD) er dokumenteret effektiv i høj permeable aflejringer. ERD er også opstartet på en række lav permeable morænelerslokaliteter. I denne afhandling er udviklingen af reduktiv dechlorering af chlorerede ethener (og ethaner) undersøgt i moræneler med henblik på at opnå en bedre forståelse af nedbrydnings processer samt evaluere ERD som oprensningsmetode i moræneler. Udviklingen af nedbrydning i moræneler er undersøgt på to lokaliteter: en hvor der gennem fire årtier er sket naturlig omsætning af chlorerede ethener og ethaner (Vadsbyvej), og en anden hvor ERD har fundet sted i fire år (Gl. Kongevej). Nedbrydningen af chlorerede ethener og ethaner i lermatricen og i indlejrede sand linser og slirer blev undersøgt ved delprøvetagning af intakte kerneprøver og grundvandsprøvetagning. En integreret fremgangsmåde med brug af kemisk analyse, molekylære microbielle værktøjer og stofspecifik isotop analyse (CSIA) blev benyttet. Resultaterne fra fuldskala oprensningen blev sammenstillet med resultaterne fra en fuld skala oprensning, hvor der blev injiceret donor og bakterier ved gravitation (Sortebrovej, Danmark).

Undersøgelserne af naturlig nedbrydning af chlorerede ethener og ethaner i moræneler viste et komplekst system hvor diffusion, biotiske og abiotiske processer forløb parallelt. Den diskretiserede prøvetagning kombineret med brug af kemisk analyse, microbielle molekulære værktøjer og CSIA var nødvendig for at identificere både biotiske og abiotiske nedbrydnings zoner. Reduktiv dechlorering af TCE til cis-DCE fandt gennem hele sektioner (op til 3 m), hvor områder med delvis nedbrydning til VC og ethen var udviklet mere sporadisk, sandsynligvis på grund af begrænset tilstedeværelse af *Dehalococcoides* med *vcrA*. Undersøgelsen viser, at der er potentiale for udvikling af nedbrydning over større dybder i moræneler.

Når ERD implementeres i lavpermeable aflejringer, er en af de største udfordringer at opnå en god kontakt mellem elektron donor, bakterier og forureningsstoffer. To injektionsmetoder blev derfor testet ved injektion af substrat lignende sporstoffer: hydraulisk frakturering med efterfølgende injektion ved gravitation og ”direct push” injektion. Undersøgelserne viste, at det var muligt at lave en hydraulisk sprække i 3 m under terræn (u.t.). Det var ikke muligt at lave horisontale hydrauliske frakturer mellem 6 og 9.5 m u.t. Ved ”direct push” injektion var muligt at lave tætliggende horisontale sporstoftilsætninger (10 og 25 cm afstand) fra 2.5 til 9.5 m u.t.

Det organiske substrat og bakterier blev primært spredt i naturlige sand linser, sand slirer og blødt moræneler efter injektion ved gravitation og ”direct push”. Ved ”direct push” injektion blev der yderligere set spredning i naturlige eller inducerede sprækker. I modsætning til observationerne ved injektionstestene blev der ikke i alle tilfælde observeret sprækker for hver 25 cm (injektions interval). Efter fire års oprensning med ERD i moræneler blev der fundet en meget heterogen udvikling af nedbrydning i lermatricen. I nogle områder var nedbrydningen begrænset til smalle områder omkring blødt moræneler, sand slirer og sand linser, hvorimod nedbrydning havde udviklet sig gennem hele profiler andre steder (op til 1.8 m på Gl. Kongevej). Der skete kun mindre eller ingen omsætning i de ubehandlede områder. På Gl. Kongevej blev nedbrydningen i lermatricen dokumenteret ved berigede isotop fraktioner for TCE og cis-DCE og tilstedeværelsen af *Dehalococcoides* med det funktionelle gen *vcrA* i lermatricen. Nedbrydningen i lermatricen var ikke så fremskreden som i grundvandet. Dette viser, at det er nødvendigt at undersøge udviklingen i lermatricen for at danne et realistisk billede af oprensningen.

De korteste oprensningstider blev fundet for områder, hvor nedbrydningen fandt sted i hele lermatricen (ca. 20 år). I områder, hvor nedbrydning var begrænset til områder omkring sand slirer og sand linser, var oprensningstiden væsentlig længere (op til 170 år). Dette viser, at det er nødvendigt, at der sker omsætning i hele lermatricen, hvis ERD skal være en rentabel oprensningsmetode i moræneler.

LIST OF CONTENTS

1 INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Many contaminants are emerging in the environment on a worldwide scale. These contaminants pose a risk to living creatures through their presence in air, surface waters, groundwater, soil, etc. As a result of widespread and insufficiently controlled use, chlorinated solvents are among some of the most frequently found contaminants in groundwater in the industrialized world incl. Denmark (DEPA, 2006) and the United States (U.S. Department of the Interior and U.S. Geological Survey, 2006). Some of the chlorinated solvents and their degradation products are toxic and carcinogenic (U.S. Department of Health and Human Services, 2006) and are therefore unwanted in our water resources.

In Denmark the glacial deposit clay till is found as the upper geological unit in 40% of the country (Gravesen et al., 2006). Clay till is also predominant in other parts of Europe and North America (Levin, 2003). Clay till is a low permeability, poorly sorted (diamict), sedimentary deposit which contains a range of natural fractures and embedded sand lenses and stringers (Kessler et al., 2012; Klint, 2001). These natural features create preferential transport pathways for contaminants such as chlorinated solvents. When chlorinated solvents are spilled on a fractured medium, they will be transported downwards as a dense non-aqueous phase liquid (DNAPL) through these preferential pathways due to their immiscibility with and higher density than water. Due to the concentration gradient between fractures and the sediment matrix dissolved chlorinated solvents will diffuse into the sediment matrix (Falta, 2005; Reynolds and Kueper, 2002). The initial diffusion is fast and the resulting mass in the clay till matrix will serve as a long-term source to contamination of the underlying aquifer due to back diffusion from the matrix to the infiltrating groundwater (Chambon et al., 2011; Parker et al., 2008; Chapman and Parker, 2005). Remediation technologies for mass removal of chlorinated solvents in source zones in low permeability settings are therefore needed.

An often applied *ex situ* technology is excavation where the contaminated sediment is removed and treated elsewhere, but due to depth or location of the contamination (e.g. under buildings) this is not an option at many sites. *In situ* remediation technologies where the contamination is treated on-site through mass removal or mass transfer are therefore needed. An *in situ* remediation technology that has been found efficient in high permeability settings is enhanced reductive dechlorination (ERD) (e.g. Suthersan et al., 2011; Scheutz et al., 2008; Lookman et al., 2007; Major et al., 2002; Ellis et al., 2000) where the right conditions for complete reductive dechlorination to the harmless ethene are created through biostimulation and/or bioaugmentation. A central limitation when remediating low permeability sediment is the reduced ability to create contact between the reactant, bacteria and the contaminated media. Currently knowledge pertaining to the development of degradation in the clay till matrix is limited and remediation timeframes are therefore difficult to estimate (Chambon et al., 2010).

1.2 Research Objectives

The aim of the present PhD study has been to investigate the development of reductive dechlorination of chlorinated solvents in clay till to obtain knowledge of degradation processes and to evaluate ERD as a remediation technology in clay till. More specific objectives have been:

- To conduct a field test of enhanced injection methods (direct push injection with GeoProbe® and hydraulic fracturing followed by gravitational injection) to investigate the possibility to overcome diffusion limitations of the low permeability sediment. (Christiansen et al., I and II)
- To investigate the occurrence and distribution of natural degradation processes of chlorinated ethenes and ethanes in a clay till matrix and high permeability features such as sand lenses and sand stringers. (Damgaard et al., III; Chambon et al., V)
- Evaluate the effect of ERD in clay till. This included an evaluation of the spreading of electron donor in the clay till sediment, development of degradation in the clay till, mass removal calculations and side effects of ERD in clay till. (Damgaard et al., IV)

1.3 Outline for the PhD Thesis

The structure of the thesis is as follows. In Chapter 2 the transport of chlorinated ethenes and ethanes in a low permeability setting is described followed by an overview of the different degradation paths of chlorinated ethenes and ethanes. In Chapter 3 ERD as a remediation technology in clay till is described. This includes a description the concept of biostimulation and bioaugmentation and the geological variations influencing the delivery of organic substrate (biostimulation) and bacteria (bioaugmentation) in the subsurface. Chapter 4 provides an overview of parameters measured when evaluating degradation of chlorinated ethenes and ethanes in a low permeability setting. Special attention is paid to the advanced molecular microbial tools and CSIA. In Chapter 5 the results after implementation of ERD in clay till are discussed. This includes a description of the spreading of donor and bacteria in the clay till followed by the development of degradation in both high permeability features and the clay till matrix. Finally, an evaluation of ERD in clay till is made with focus on mass removal, timeframes and side effects. The conclusions of the PhD study are presented in Chapter 6. Suggestions for future research within this area are made in Chapter 7. The thesis is based on four scientific papers and one technical note. These are found in Chapter 9.

2 CHLORINATED SOLVENTS

2.1 USE OF CHLORINATED SOLVENTS

Chlorinated solvents such as tetrachloroethene (PCE) and trichloroethene (TCE) have been widely used in industry for dry-cleaning and as metal degreasing agents. The consumption increased up to the 1970. Since then rising environmental awareness and legislation have decreased the use (Doherty, 2000a; Doherty, 2000b). As several of the chlorinated compounds are suspected carcinogenic (U.S. Department of Health and Human Services, 2006) they are only allowed in very low concentrations in drinking water (Danish drinking water criteria (DWC) are given in Table 1).

Table 1: Characteristic parameters at 25 ºC for chlorinated ethenes and ethanes

	Solubility[1]	**Density[1]**	**K_H[1]**	**DWC[2]**
	mg/L	**kg/L**	-	**µg/L**
Chlorinated ethanes				
1,1,1-Trichloroethane (1,1,1-TCA)	4394	1.34	0.70	1
1,1-dichloroethane (1,1-DCA)	4767	1.18	0.23	1
Chloroethane (CA)	5700	0.90	0.46	1
Chlorinated ethenes				
Tetrachloroethene (PCE)	240	1.63	0.72	1
Trichloroethene (TCE)	1400	1.47	0.39	1
1,1-dichloroethene (1,1-DCE)	3300	1.22	1.10	1
1,2-cis-dichloroethene (cis- DCE)	3500	1.27	0.17	1
1,2-trans-dichloroethene (trans- DCE)	6300	1.25	0.38	1
Vinylchloride (VC)	2800	0.92	1.10	0.2

1Kjeldsen and Christensen (1996) 2 DEPA (2011)

2.2 SOURCE DEVELOPMENT OF CHLORINATED SOLVENTS IN CLAY TILL

Chlorinated compounds are immiscible with and have a higher density than water (Table 1), which means that they will be transported as a dense non-aqueous phase liquid (DNAPL) down through the subsurface. When spilled on low permeability media, such as clay till, natural fractures, sand lenses and stringers will provide fast transportation paths for the DNAPL both horizontally and vertically (Figure 1, a). Since the water solubility is relatively high (Table 1) dissolution of the compounds in groundwater will occur. The concentration gradient between the dissolved phase in the fractures, sand lenses or sand stringers and the clay matrix will cause diffusion of the dissolved chlorinated solvents into the soil matrix (Figure 1, b). The diffusion of dissolved contaminants into the matrix is initially fast (Falta, 2005; Reynolds and Kueper, 2002). The sorption of the contaminants in clay till is higher than in other sediments with similar content of organic carbon (in clay till TCE range from 0.62-0.96, cis-DCE 0.17-0.82 and VC 0.12-0.36 L/kg, Lu et al., 2011). When DNAPL is no longer present the mass present in the matrix will back-diffuse into the infiltrating groundwater whereby the contamination in the clay till matrix will serve as a long-term source to contamination of the underlying aquifer (Figure 1, c) (e.g. Parker et al., 2008; Chapman and Parker, 2005; Parker et al., 2004; Parker et al., 1997; Parker et al., 1994).

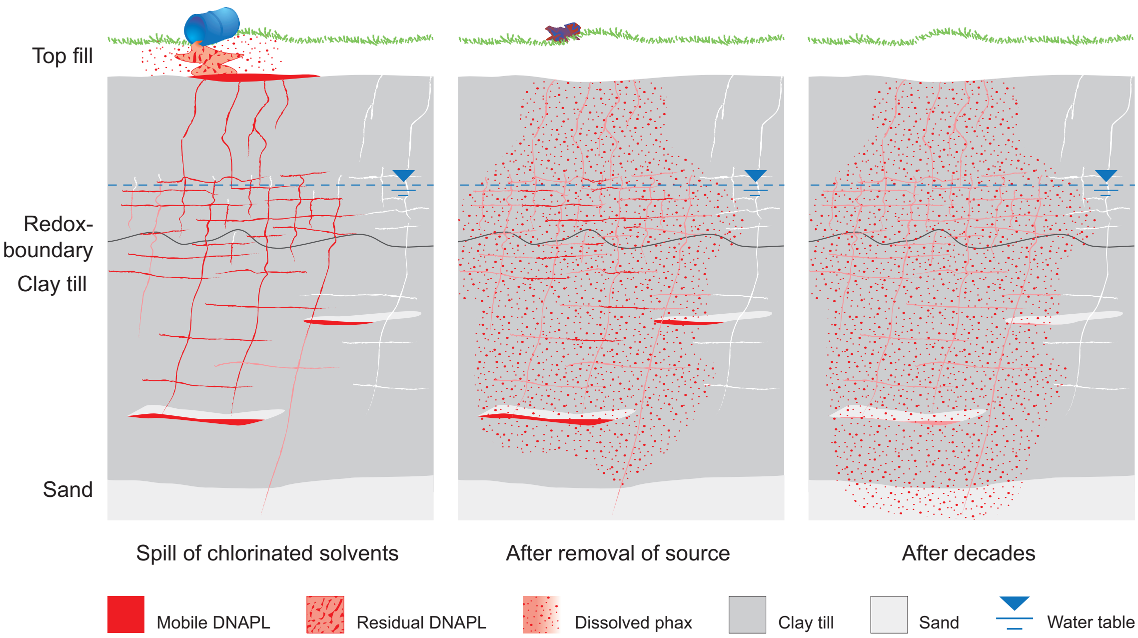

Figure 1: (a) DNAPL spill at clay till overlaying a high permeability aquifer. DNAPL is initially transported in fractures, sand lenses and stringers. (b) Dissolved phase is transported into the clay till matrix by diffusion. (c) DNAPL is no longer present. Concentrations levels in the clay till matrix exceed the concentration in fractures, sand lenses and stringers, resulting in back diffusion of the contaminants into the infiltrating groundwater. Contaminants are transported to the underlying aquifer. Modified from Jørgensen et al., 2010.

2.3 Degradation of chlorinated solvents

In an aquatic environment chlorinated ethenes and ethanes can be degraded by abiotic and biotic pathways depending on the conditions and the microbes present in the subsurface. The biotic degradation of chlorinated ethenes is well documented in literature, whereas the knowledge about degradation of chlorinated ethanes is more limited (review by Scheutz et al., 2011).

2.3.1 Chlorinated ethenes

The most studied biotic degradation path of chlorinated ethenes is anaerobic dechlorination (e.g. Aulenta et al., 2006; Bradley, 2003; Duhamel et al., 2002;

Hendrickson et al., 2002; US EPA, 1998; Vogel et al., 1987). In contrast natural abiotic degradation pathways have been less investigated. An overview of the biotic and abiotic degradation pathways of chlorinated ethenes under anaerobic conditions is shown in Figure 2.

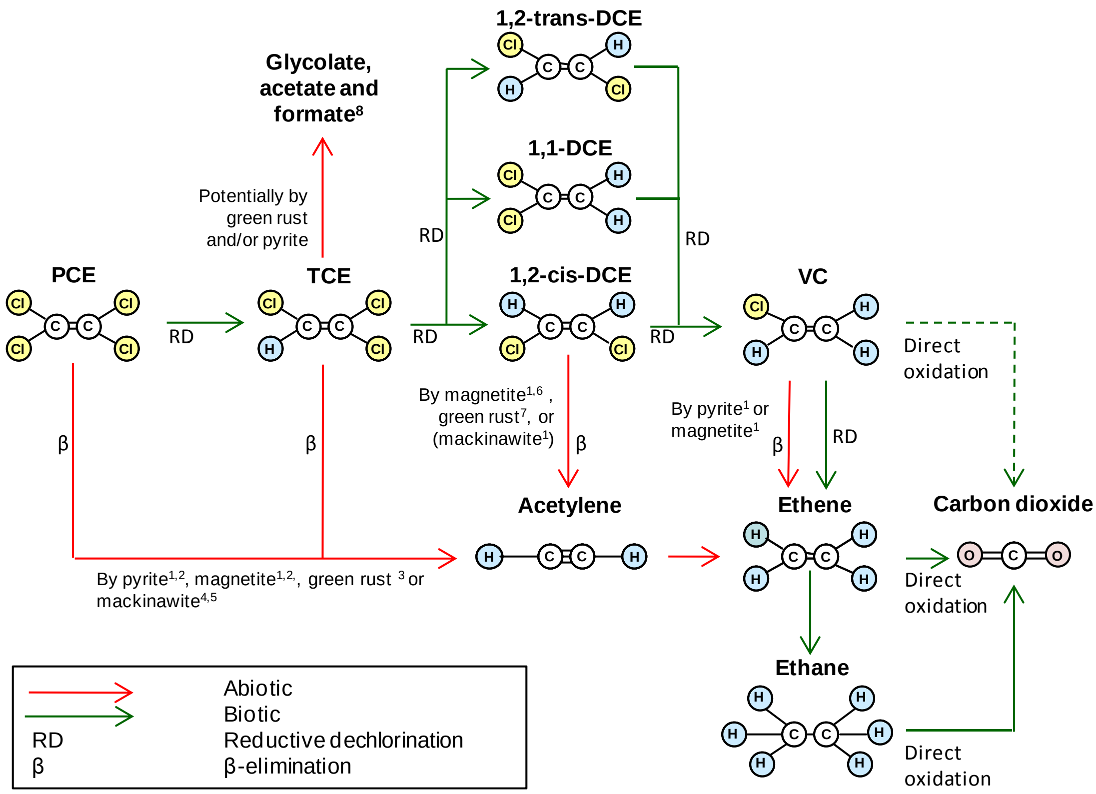

Figure 2: Overview of anaerobic biotic and abiotic degradation processes of chlorinated ethenes. Dashed lines marks possible degradation paths. 1) Lee and Batchelor, 2002a; 2) Weerasooriya and Dharmasena, 2001; 3) Lee and Batchelor, 2002b; 4) Butler and Hayes, 1999; 5) Kennedy et al, 2006; 6) Ferry et al, 2004; 7) Jeong et al, 2011; 8) Darlington et al, 2008.

Biotic degradation

Chlorinated ethenes can under anaerobic conditions be dechlorinated all the way to ethene or ethane (Figure 2). The reductive dechlorination process can proceed either as a cometabolic or a halorespiring dechlorination. Microbes only obtain energy in the halorespiring process where the chlorinated compound is used as a terminal electron acceptor (review by Middeldorp et al., 1999).

Several different microbes are found to dehalorespire PCE and TCE to cis-DCE (*Dehalobacter restrictus*, *Desulfitobacterium or desulfuromonas chloroethencia*) whereas the only microbes found that degrades cis-DCE to VC and ethene are *Dehalococcoides spp.* (Friis, A.K., 2006; Sung et al., 2006; Cupples et al., 2003; Hendrickson et al., 2002; Middeldorp et al., 1999). *Dehalococcoides* containing the gene *tceA* respire TCE and cis-DCE (Johnson et al., 2005; Seshadri et al., 2005) whereas *Dehalococcoides* with the VC reductase genes *vcrA* or *bvcA* can respire cis-DCE and VC (Sung et al., 2006; Müller et al., 2004). Reductive dechlorination occurs when specific anaerobic bacteria are present at the contaminated site along with an organic compound serving as an electron donor. The bacteria carrying out dehalorespiration of chlorinated ethenes use hydrogen as electron donor (Fennell and Gossett, 1998; Lee et al., 2007; MaymoGatell et al., 1997). He et al. (2002) through showed that acetate can also be an effective electron donor for the dechlorination from cis-DCE to VC.

The higher chlorinated ethenes (PCE and TCE) are most oxidized and therefore the reductive degraders yield more energy than the lower chlorinated ethenes (Vogel et al., 1987). As a result cis-DCE and VC potentially have a risk of accumulating in the system. Accumulation of cis-DCE and VC can also be a consequence of lack of the specific degraders able to carry out complete degradation (US EPA, 1998), due to inhibition between the chlorinated ethenes (e.g. Popat and Deshusses, 2011; Sabalowsky and Semprini, 2010; Yu et al., 2004) or due to inhibition by co-contaminants such as chloroform or 1,1,1-TCA (Duhamel et al., 2002). It has been proposed that high concentrations of chlorinated compounds (750μmol/L of both cis-DCE and VC, Yu and Semprini, 2004) can have an inhibitory or toxic effect on dechlorinating cultures (Yu and Semprini, 2004; Duhamel et al., 2002).

The reductive dechlorination is very sensitive and requires an anaerobic environment. Chlorinated ethenes have been found to undergo reductive dechlorination under iron and sulfate reducing conditions (Wei and Finneran, 2011; Aulenta et al., 2007; Heimann et al., 2005). Hoelen and Reinhard (2004) showed that complete dechlorination of PCE to ethene could take place at concentration >100 mg/L of sulfate. Older studies suggest that degradation of VC to ethene is found to proceed at the highest rate at methanogenic conditions (Chapelle, 1996; Yang and McCarty, 1998).

VC can be degraded by aerobic oxidation to CO_2 (Verce et al., 2000). In the oxidation process VC acts as the electron donor while natural oxidized compounds serve as electron acceptors. Anaerobic oxidation of cis-DCE and VC has been discussed among researchers and is therefore illustrated by a dashed line in Figure 2. Anaerobic oxidation has been documented to take place (Smits et al., 2011). Cox et al. (2010) and Gossett (2010) have though suggested that the lack of VC at some sites can be due to aerobic oxidation at low oxygen concentrations.

Abiotic degradation

Abiotic degradation of chlorinated ethenes has been proposed to have two pathways: abiotic reductive dechlorination and β-elimination. In abiotic reductive dechlorination a chlorine atom is replaced by a hydrogen atom whereas acetylene is formed by β-elimination. Acetylene is more commonly found as degradation product in abiotic degradation experiments and is therefore considered the most pronounced abiotic degradation path (e.g. Liang et al., 2009; Lee and Batchelor, 2002; Butler and Hayes, 1999). Naturally occurring iron-containing minerals, such as FeS formed as a result of iron and sulfate reduction, have been found to serve as reductants for abiotic degradation of chlorinated ethenes (Figure 2). This suggests a abiotic degradation mediated by biotically produced iron minerals. Combined abiotic and biotic degradation of TCE and cis-DCE was found to proceed in microcosms with iron rich sediment by Darlington et al. (2008).

2.3.2 CHLORINATED ETHANES

Chlorinated ethanes can be degraded by a range of abiotic and biotic degradation paths. An overview of the processes can be seen in Figure 3.

Biotic degradation

Chlorinated ethanes can be biotically degraded by reductive dechlorination, aerobic cometabolic degradation and direct oxidation. Reductive dechlorination of 1,1,1-TCA has been observed under sulphate reducing and methanogenic conditions (review by Scheutz et al., 2011). Similar to the reductive dechlorination of chlorinated ethenes one chlorine atom can be substituted by a hydrogen atom when an electron donor and a catalyst are present (Figure 3). The reductive dechlorination can take place cometabolically or as energy releasing dehalorespiration. Reductive dechlorination by dehalorespiration is found to achieve significantly higher dechlorination rates (review by Scheutz et al., 2011). The reductive dechlorination of 1,1,1-TCA has been observed to be faster than the reductive dechlorination of 1,1-DCA (review by Scheutz et al., 2011).

The first isolated strain that was found to carry out dehalorespiration of 1,1,1-TCA and 1,1-DCA was TCA1 which was found to be related to the *Dehalobacter restrictus* (Sun et al., 2002). Grostern and Edwards (2006) have since then reported the first mixed culture (*Dhb*-TCA) that can dehalorespire 1,1,1-TCA and 1,1-DCA yielding CA as terminal product. CA can possibly be degraded by direct oxidation similar to VC. However, the process is not very well documented (review by Scheutz et al., 2011).

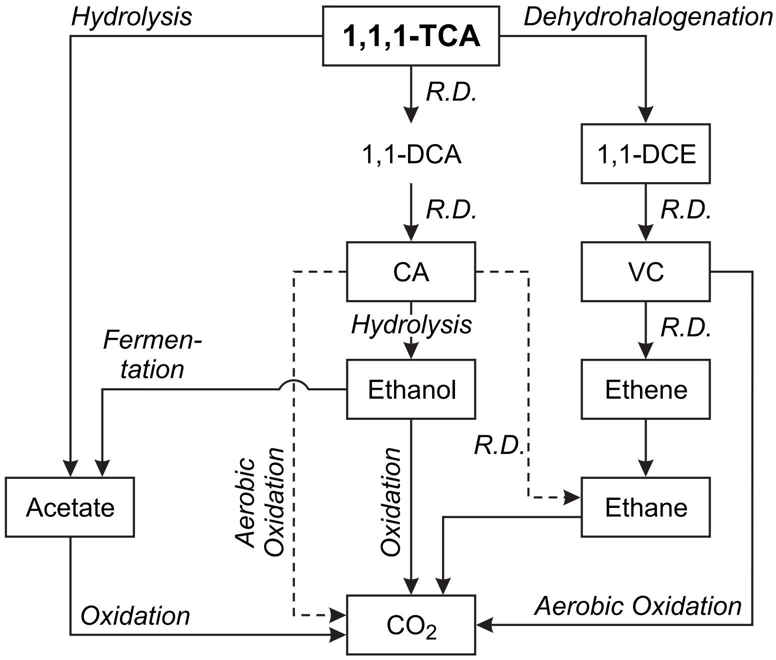

Figure 3: Abiotic and biotic degradation paths for 1,1,1-TCA under aerobic and anaerobic conditions. R.D. denotes reductive dechlorination and dashed lines marks potential but not documented pathways. Figure from Scheutz et al. (2011) with permission.

Abiotic degradation

Chlorinated ethanes can be abiotically degraded by three different processes: hydrolysis, dehydrohalogenation and abiotic reductive dechlorination. The first two are relatively well investigated whereas reductive degradation catalysed by reactive metals is not very well understood (review by Scheutz et al., 2011). 1,1,1-TCA has been observed to be abiotically degraded by FeS in laboratory studies (Gander et al., 2002; Butler and Hayes, 2000). 1.1-DCA has been found to account for 4-6% of the 1.1.1-TCA degraded but the degradation path is not very well documented (Gander et al., 2002).

Both hydrolysis and dehydrohalogenation can proceed under oxic and anoxic conditions. When 1,1,1-TCA is hydrolysed a chlorine group is stepwise exchanged with a hydroxyl group until acetate is formed. In abiotic dehydrohalogenation of 1,1,1-TCA one chlorine and a hydrogen is removed from each their carbon atom whereby 1,1-DCE is formed. Hydrolysis and dehydrohalogenation will proceed simultaneously, however as the hydrolysis is found to be a factor of 5 faster, more acetate is formed (review by Scheutz et al., 2011).

Inhibitory effect on degradation of chlorinated ethenes

1,1,1-TCA have an inhibitory effect on methanogenesis and acetogenesis. As these processes possibly supports *Dhc* in reductive dechlorination of chlorinated ethenes 1,1,1-TCA is thought to have an inhibitory effect (review by Scheutz et al., 2011). Duhamel et al. (2002) have also found that 1,1,1-TCA in concentrations of 700 µg/L can completely inhibit dechlorination of cis-DCE and VC in batch reactors with KB-1 containing two strains of *Dhc*. Contrary, Chan et al. (2011) found that 1,1-DCA did not have an inhibitory effect on the reductive dechlorination of chlorinated ethenes.

3 ERD AS A REMEDIATION TECHNOLOGY IN CLAY TILL

In situ ERD is considered a cost effective and environmentally friendly method to remediate sites contaminated with chlorinated solvents (e.g. Lemming et al., 2010). For ERD to occur, the conditions have to be anaerobic, the necessary bacteria for complete degradation to harmless ethene have to be present and the right conditions for the microbes to grow have to be achieved. This can be obtained by addition of a carbon source (biostimulation) and specific degraders (bioaugmentation) to the subsurface. When ERD is applied in a low permeability setting one of the challenges is to establish the necessary contact between the contamiants, the carbon source and bacteria to obtain reasonable timeframes for the remediation (Chambon et al., 2010). It is therefore important to consider the geological characteristics (fractures, sand lenses and stringers) as these initially have governed the transport of the contaminants and therefore also control how amendments subsequently can be distributed in the subsurface. This leads to two practical issues that have to be considered when applying ERD in clay till: clay till characteristics and delivery of amendments in the subsurface. In the following chapter the concept of biostimulation and bioaugmentation will be described followed by a description of clay till characteristics and injection methods applied in clay till.

3.1 BIOSTIMULATION

Biostimulation of reductive dechlorination is carried out by adding organic substrates as a carbon source to the subsurface. Biostimulation serves two purposes: reducing the environment to make the conditions more favorable for reductive dechlorination and to make sure there is electron donor available for the reductive dechlorination. When organic substances are injected into the clay till a series of fermentation and respiration processes will proceed simultaneously (Figure 4). Fermentation processes will produce electron donor and respiration processes will create more reduced conditions for the reductive dechlorination to proceed. A range of different physical, chemical and microbial factors in the subsurface controls which processes that will proceed and in which order they will proceed when organic substrates are added to the subsurface.

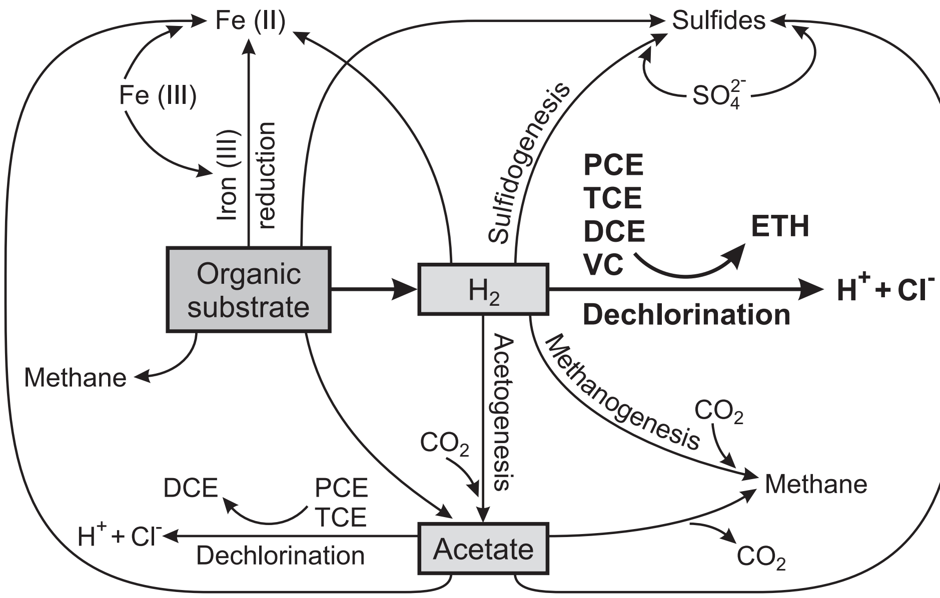

Figure 4: Interaction between redox, fermentation and degradation processes. Modified from Fennell and Gossett (2004).

In reductive dechlorination the preferred substrate for the dechlorinators is hydrogen (Lee et al., 2007; Fennell and Gossett, 1998; MaymoGatell et al., 1997). However, He et al. (2002) found that also acetate can be an effective electron donor to support dechlorination of cis-DCE to VC. Hydrogen can be produced by fermentation of organic substrates meaning that these can be used to stimulate the reductive dechlorination. However, other respiratory organisms (such as methanogens, acetogens, sulfate- and nitrate reducers and Fe(II) and Mn(IV) reducers) (Aulenta et al., 2007; Aulenta et al., 2006) in the subsurface also use hydrogen and acetate as electron donors. There will therefore be competition for these compounds when present in the subsurface (Figure 5).

Several different organic substrates have been tested in field and laboratory studies as hydrogen donor for reductive dechlorination (e.g. lactate, formate, propionate, Azizian et al., 2010 and others, ESTCP, 2004). The rate and extend of H_2 production varies among different donors whereby the effect on the dechlorination also differs (review by Aulenta et al., 2006).

The donors used for ERD at low permeability sites are listed in Table 2. The electron donors are relatively immobile (ESTCP, 2004) and slowly fermentable leading to a continuous production of hydrogen. This is preferable in clay till where matrix transport is diffusion limited. Newman Zone™ and EOS® are vegetable oil products whereas molasses is a left over product from refined sugar production. The commercial products Newman Zone™ and EOS® both contain ~4% fast hydrogen releasing lactate.

Table 2: Overview of electron donors used in ERD at low permeability sites.

Site	Donor	Reference
Rugårdsvej, Denmark	Newman Zone™	Scheutz et al., 2010
Sortebrovej, Denmark	EOS®	Manoli et al., 2012
Gl. Kongevej, Denmark	Organic Molasses	Damgaard et al., IV

The necessary amount of electron donor needed to obtain the wanted processes can be estimated from the content of inorganic electron acceptors in the subsurface and the soluble and sorbed amount of contaminants. For further recommendation on the choice and calculation of the amount of donor see ESTCP (2004) and ESTCP (2010).

3.2 BIOAUGMENTATION

Bioaugmentation involves injection of a bacterial community with a certain composition (containing *Dhc*) known to carry out degradation of chlorinated ethenes and ethane. At some sites *Dhc* are naturally present which means that biostimulation with organic substrate can enhance the proliferation, but at other sites it is necessary to add the specific degraders (bioaugmentation) to ensure that complete degradation will occur (Lu et al., 2006; Hendrickson et al., 2002; Fennell et al., 2001;). Several commercial cultures containing the dechlorinating culture *Dhc spp.* are available. Cultures used in ERD in clay till are listed in Table 3.

Table 3: Dechlorination cultures used in ERD applications in clay till.

Site	Culture	Company	Reference
Rugårdsvej, Denmark	KB-1®	SiREM, Guelph, Ontario, Canada	Scheutz et al., 2010
Sortebrovej, Denmark	KB-1®	SiREM, Guelph, Ontario, Canada	Manoli et al., 2012
Gl. Kongevej, Denmark	Dechlorinating culture	Bioclear, Netherlands	Damgaard et al., IV

As different bacteria are known to carry out different steps of the dechlorination (Friis, 2006; Middeldorp et al., 1999) a number of different bacterial species including *Dhc* spp. and *Dhb*. Spp. are found in the microbial cultures used for bioaugmentation e.g. in KB-1® (Duhamel and Edwards, 2006; Duhamel et al., 2004; Duhamel et al., 2002). The dechlorinating culture from BioClear contained from 0.01% to 2.6% *Dhc* of the total population of bacteria (2.8×10^9 cells/mL) (Miljøkontrollen, 2006a).

KB-1® has been proven to enhance reductive dechlorination in laboratory studies (e.g. Friis et al., 2007) and *in situ* bioremediation in high (e. g. Scheutz et al., 2008; Peale et al., 2008; Major et al., 2002) and low permeability aquifers (Manoli et al., 2012; Scheutz et al., 2010). The dechlorinating culture from

BioClear was proven effective in batch experiments with sediment from Gl. Kongevej prior to implementation of the remediation (Miljøkontrollen, 2006b). The culture was also proven efficient in low permeability clay till (Damgaard et al., IV).

Several factors can influence the proliferation / performance of dechlorinators after bioaugmentation, e.g. groundwater pH (Middeldorp et al., 1999), temperature (Friis et al., 2007; Heimann et al., 2007), donor (e.g. Aulenta et al., 2007; Azizian et al., 2010; Heimann et al., 2007; He et al., 2002), competition for donor (discussed in section 3.1) and inhibition by contaminant concentration levels or presence of co-contaminants (discussed in section 2.3).

3.3 CLAY TILL CHARACTERIZATION

The main initial transportation path for the chlorinated solvents in clay till is sand lenses, stringers and fractures (explained in section 2.2). In heterogeneous sediments such as clay till there are large variations in fracture depths, apertures and spacing in clay till. Knowledge of expected fracture types and frequencies can be obtained by understanding the glacial processes that have created the landscape and the processes that have created the fractures in the clay till (Klint, 2001). The clay till can hereby be classified and, hence, information on fractures and other features can be obtained.

Till is defined as a poorly sorted deposit of glacial origin. Clay till consists of clay, silt, sand, gravel and rocks, where clay, silt and sand usually constitute as the till matrix (Krüger, 2000). The clay content in clay till in Denmark, ranges from few percent to 35% (Houmark-Nielsen and Kjær, 2005). Depending on the various glacial environments in which the till was deposited, clay till can be classified as: basal clay till, flow till and melt-out till. The glacial environment has great influence on the different fracture types and frequencies of fractures that can be expected in tills. The most commonly found till in Denmark is basal clay till (Houmark-Nielsen and Kjær, 2005), which is deposited in the sub-glacial environment under a glacier as deformation till, lodgement till or melt-out till. Till can also be deposited on top of the glacier where sediment due to melting of the ice falls down as melt-out or flow till (glacier-marginal or supra-glacial environment) (Krüger, 2000).

3.3.1 FRACTURES IN CLAY TILL

Fractures in clay till can be divided into 3 main types of fractures (Klint, 2001): glacial tectonic fractures, contraction fractures and neo-tectonic fractures.

Glacial tectonic fractures are systematically oriented fractures formed by the ice movement, and are therefore generally restricted to basal clay till. Basal clay tills can be classified as type A, when deformed during high pore water content, and type B, when formed during well-drained conditions. Type A is usually massive and unfractured but can contain hydro-fractures. In type B tills, sub-horizontal shear fractures, extension fractures or conjugation shear fractures can be expected. The four glacial tectonic fractures have been defined by Klint (2001):

- *Hydro-fractures*: Fractures created during bad drainage conditions where pore water pressure has resulted in water or water-filled sediment intrusion.
- *Sub-horizontal shear fractures*: Sub-horizontal shear fractures are usually found throughout the clay till with a tendency to increased frequency towards the bottom as they are formed along the till in the deforming bed. Often they have a weak slope (0-20º) to or contrary to the ice movement direction (Klint, 2008).
- *Extension fractures*: Extension fractures are usually found parallel to the ice movements with a slope of 80-90º.
- *Conjugating shear fractures*: The fractures have a slope of 90-60º with an orientation perpendicular to the ice movement direction.

The drainage conditions between the clay till and the underlying sediment have also been found to influence the depth and intensity of vertical glacial fractures. When the conditions are well drained the size and intensity of fractures are generally higher than in tills overlying poorly drained deposits (Klint, 2001).

Contraction fractures are formed when the sediment dries out (desiccation fractures) or by freeze/thaw processes. Thus, these fractures are irregularly vertically oriented. As the processes creating the contraction fractures are usually related to the upper part of the sediment, the fracture number decreases with depth. The maximum penetration depth is considered to be the redox boundary.

Neo tectonic fractures are created by decompression when the glacier melted or during earthquakes. Neo-tectonic fractures are rare and less investigated than the other fracture types (Klint, 2008).

3.3.2 Sand lenses and stringers in the clay till

Sand lenses and stringers with various thicknesses and distribution are common in clay tills. These permeable features in the clay till can act as transportation paths for contaminants and are therefore of high importance when working in clay till. Sand lenses and stringers can be identified in boreholes, but the distribution and/or connectivity is still rather uninvestigated. Kessler et al. (2012) found that investigations of open cross sections in a gravel pit provided data for classification of five different sand features (sand layers, sand sheets, sand bodies, sand pockets and sand stringers) with varying horizontal extend, thickness, anisotropy and vertical spacing. Investigation of the statistical distribution and connectivity is still ongoing (Kessler et al., 2012). In the present study sand features are referred to as sand lenses and sand stringers, where sand lenses are well defined sand layers of more than a cm whereas sand stringers defines thin few mm thick sand features.

3.4 Delivery methods

Several techniques have been used to inject donor and bacteria in low permeability settings. For clay tills specifically these include: hydraulic fracturing followed by gravitational injection (Scheutz et al., 2010), gravitational injection in screened wells (Manoli et al., 2012) and direct push delivery by GeoProbe® (Damgaard et al., IV).

Hydraulic fracturing, direct push delivery and pneumatic fracturing are enhanced injection techniques where the presence of sand lenses (hydraulic fracturing) and fractures (direct push delivery and pneumatic fracturing) in the subsurface is increased whereas natural sand lenses, stringers and fractures are used to spread amendments in the sub surface by gravitational injection. Pneumatic and hydraulic fracturing has been commercially used for 15-20 years. However, direct documentation below 5 m was missing (Christiansen, 2010). Test of enhanced delivery methods including pneumatic fracturing (Figure 4, A), direct push delivery by GeoProbe® (Figure 4, B) and hydraulic fracturing with subsequent gravitational injection (Figure 4, C) was therefore carried out in clay till by injection of amendment-comparable tracers (Christiansen et al., 2008; Christiansen et al., I; Christiansen et al., II). The spreading of tracers were investigated by excavation (Figure 4, D) and coring.

In the following the methods applied for implementing ERD in clay till will be described based on the enhanced injection tests carried out in clay till; direct push delivery and hydraulic fracturing followed by gravitational injection (Christiansen et al., I; Christiansen et al., II). A conceptual illustration of the methods and the expected spreading in the low permeability setting can be seen in Figure 5.

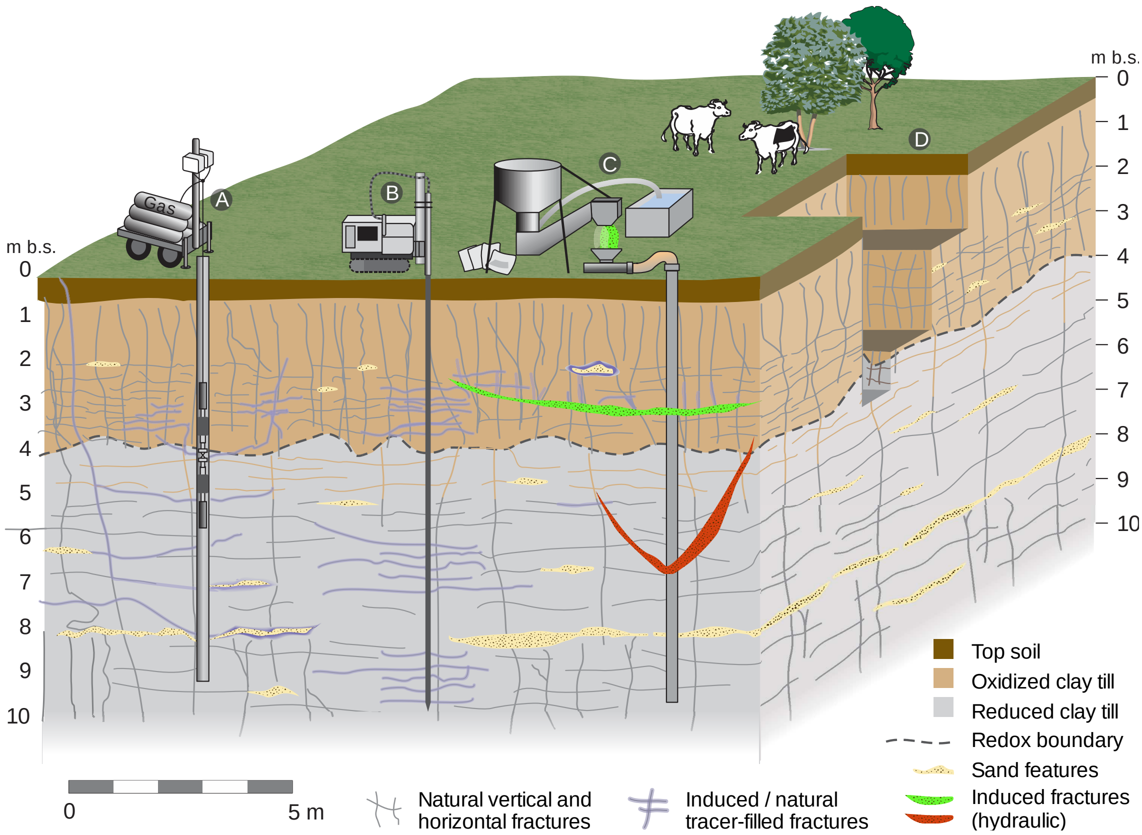

Figure 5: Conceptual illustration of A) pneumatic fracturing, B) direct-push delivery, C) hydraulic fracturing and D) an excavation. Modified from Christiansen (2010), with permission.

3.4.1 Direct push delivery

Direct-push delivery is usually carried out using a drill-rig (e.g. a GeoProbe®) to push an injection probe down through the subsurface (Figure 4, B). When the desired depths are reached, injection of substrates is performed. The injection is done using pressure whereby the substrate is distributed in natural fractures, sand stringers and lenses or a fracture is created (Christiansen et al., II).

A field test of direct push delivery was carried out at a site in Vadsby, Denmark to investigate the distribution of amendment-comparable tracers in clay till (Christiansen et al., I and II). Three tracers were used: brilliant blue (visible in daylight conditions), fluorescein (fluorescent, mobile tracer), and rhodamine WT (fluorescent, strongly sorbing tracer). The specific set up of the injection tests are described in (Christiansen et al., I and II).

Tracer injection tests of direct-push delivery showed that it was an applicable method from 0-10 m.b.s. (Christiansen et al., II). Generally the horizontal distribution radius was 1 m (though in some cases up to 2 m) and it was possible to obtain a vertical spacing of horizontal fractures of 10 and 25 cm (Christiansen et al., II)(Figure 4, B). These results reveal that amendments can be distributed well in the subsurface whereby diffusion limitations in the clay till can be overcome. Concentration profiles and pictures of the mobile fluorescent tracer used in the injection test at Vadsby also showed rapid diffusion into the clay till matrix (initially 6 cm and up to 20 cm after 3 months) from both large- and small-aperture fractures (Christiansen, 2010).

3.4.2 HYDRAULIC FRACTURING

In hydraulic fracturing a casing is installed to the depth where the hydraulic fracture is wanted. A cut into the low permeability medium is made by high water pressure to guide the direction of the fracture. Guar and water are mixed into a gel that can carry the fracturing sand. The gel is pumped into the subsurface thereby creating the pressure to create a sand lense in the low permeability setting (Figure 4, C). Injection can subsequently be carried out as gravitational injection. Gravitational injection is done via permanently screened boreholes. The substrate is added to the boreholes using low pressure (up to 0.5 bar).

A test of hydraulic fracturing in clay till was carried out in Vadsby, Denmark. Hydraulic fractures were induced at three different depths (3, 6.5 and 9.5 m.b.s.) from individual boreholes. Further, four hydraulic fractures were induced from one borehole from 6.25 to 7 m b.s. (multi-fracture) (Christiansen et al., I). Only one successful fracture was made in 3 m b.s. whereas the single fractures induced in 6.5 and 9.5 m b.s. were not located satisfactorily (even after extensive coring) and vented to the surface, respectively. The two upper fractures in the multi-fracture vented towards the surface whereas the two deeper fractures merged with an inclining direction towards the surface (~30° and 60° on the each side of the borehole) (Christiansen et al., I). These results suggest that it is difficult to create horizontally oriented hydraulic fractures from a depth between 3-9.5 m b.s. (Christiansen et al., I). Similar results were also observed in other tests of hydraulic fracturing in clay till in Denmark (Jørgensen et al., 2007; Region of Funen, 2004).

Geotechnical analysis of the sediment in different depths at Vadsbyvej suggested that the clay till was normally consolidated (Christiansen et al., I). As over consolidation supports creation of horizontal fractures this could be an explanation for the more vertically oriented propagation during the test. The test results suggest that the geotechnical properties of the sediment should be investigated before choosing hydraulic fracturing as an enhancement technology for ERD in clay till.

The same tracers as had been injected in the direct push delivery test were injected by gravitational injection into the hydraulic fractures in 3 and 6.5 m b.s. and the multi fracture. The tracers were found to spread in the created fractures, but also in natural sand stringers and lenses (Christiansen et al., I and II).

4 CHARACTERIZATION OF CHLORINATED SOLVENTS DEGRADATION IN CLAY TILL

At clay till sites the contamination is present in mobile pore water in high permeability features and immobile pore water or sorbed phase in the clay till matrix. The mobile phase in the high permeability features can be investigated by groundwater sampling, but as most of the contaminant mass is present in immobile phase in the clay till matrix (Falta, 2005; Freeze and McWhorter, 1997) both groundwater and sediment analysis is necessary. In the following chapter an overview of parameters analysed when characterising degradation of chlorinated solvents at low permeability sites will be given. Special focus will be made on the *in situ* screening and the potential of using CSIA and molecular microbial tools in the assessment of degradation of chlorinated solvents in clay till.

4.1 PARAMETERS INVESTIGATED

The degradation of chlorinated ethenes in low permeability media have been investigated in several studies. Takeuchi et al. (2011) and Damgaard et al. (III) investigated the natural degradation of chlorinated solvents in a clay aquitard and clay till respectively whereas ERD in clay till was investigated by Scheutz et al. (2010), Manoli et al. (2012) and Damgaard et al. (IV). Reductive dechlorination of chlorinated solvents is governed by several factors such as redox conditions, donor availability and presence of degraders (further described in section *2.3*). Consequently many parameters are investigated when characterizing degradation of chlorinated solvents. An overview of the parameters analysed when investigation is carried out in low permeability setting is given in Table 2.

Table 4: Overview of studies investigating degradation of chlorinated ethenes and ethanes in a low permeability media. Dark grey and light grey illustrates analysis on groundwater and sediment respectively. X marks parameters where CSIA was applied.

		Natural attenuation				ERD in clay till					
Reference		Takeuchi et al., 2011		Damgaard et al. III		Scheutz et al., 2010		Manoli et al., 2011		Damgaard et al., IV	
Study site		Yamagata Prefecture, Japan		Vadsbyvej, Denmark		Rugårdsvej, Denmark		Sortebrovej, Denmark		Gl. Kongevej, Denmark	
Geological setting		Clay aquitard		Clay till		Clay till		Clay till		Clay till	
Major contaminants		PCE		PCE, TCE, TCA		TCE		TCE		TCE, TCA	
Depth of contamination (m.b.s.)		3-7		2-15		4-10		10-20		2-8	
Geological parameters	Water content										
	Pore size distribution										
Chlorinated solvents	Chlorinated ethenes			X	X					X	X
	Chlorinated ethanes			X	X					X	X
	Ethene and ethane										
Redox sensitive parameters	ORP										
	NO_3^-										
	Dissolved Fe										
	Solid Fe(III)										
	SO_4^{2-}										
	S^{2-}										
	CH_4										
Donor availability	Volatile fatty acids (formate, lactate, propionate and acetate)										
	H_2										
	Hydrogene producing bacteria										
16SrRNA	Total										
	Dehalococcoides										
	Dehalobacter										
	Geobacter										
Functional genes	*tceA*										
	vcrA										
	bvcA										
Activity	*vcrA*										
	bvcA										

The presence of degradation products gives a direct indication of degradation (Reitzel, 2005). Thus, the distribution between mother compounds (PCE, TCE and 1,1,1-TCA) and degradation products (cis-DCE, 1,1-DCA, VC, CA, ethene and ethane) was investigated at all sites (Table 4). At Vadsbyvej and Gl. Kongevej the vertical distribution and occurrence of mother and degradation compounds were investigated *in situ* by MIP screening combined with field GC measurements and by laboratory analysis on groundwater and sediment samples. Whereas, the other locations (Rugårdsvej and Sortebrovej) only the relation between mother compounds and degradation products was investigated in groundwater and sediment samples only.

The redox conditions were investigated through measurement by analysis of natural electron acceptors present in the groundwater (NO_3^-, dissolved Fe, SO_4^{2-}, S^{2-} and CH_4 (dissolved gas)), matrix pore water (NO_3^-, SO_4^{2-} and CH_4 (dissolved gas)) and in the sediment (solid Fe(III), only Vadsbyvej)(Damgaard et al., III; Damgaard et al., IV; Manoli et al., 2012; Scheutz et al., 2010 and Takeuchi et al., 2011). At one location oxidation-reduction potential (ORP) was applied (Takeuchi et al., 2011). Christensen et al. (2000) and McMahon and Chapelle (2008) have reviewed how to asses redox processes in the subsurface. The geochemical data does not give a direct indication of degradation; however, they are essential to understand the processes taking place in the subsurface.

At Yamagata Prefecture (Takeuchi et al., 2011) the donor availability was investigated by analysis of hydrogen gas in sediment samples and the presence of hydrogen producing bacteria. At the other locations the donor availability for reductive dechlorination was investigated through analysis of volatile fatty acids (VFA, lactate, propionate, formate and acetate). VFA are formed during fermentation of organic matter/substrate (Madigan et al., 2009) and is therefore a good indicator for production of hydrogen.

4.2 *IN SITU* EVALUATION OF DEGRADATION

A standard *in situ* method used to evaluate the vertical distribution of contaminants is to take out soil samples for every 0.5 m while drilling to do *in situ* measurements by a Photo ionization detector (PID) and/or to send samples for laboratory analysis of soil concentrations. The PID measurement can be used to get an idea of the relative contaminant concentration level whereas the knowledge of the distribution between mother (PCE and TCE) and daughter products can be obtained from the soil sample analysis. However, a disadvantage of this method is that it is hard to specify what the soil sample represents as high permeability features and the clay till matrix are mixed during drilling (Damgaard et al., 2008). As the degradation is found to develop slower in the clay till matrix than in the high permeability features (Damgaard et al., IV) these results can be misleading.

Many direct push technologies are emerging which can be used to obtain high resolution samples *in situ* (Dietrich and Leven, 2009). One that has been extensively used in low permeability media to assess the vertical distribution of contaminants in the sub-surface is MIP measurements (e.g. Damgaard et al., III and IV; Capital Region of Denmark). MIP is a direct push tool where a heated probe (100-120°C) is pushed down through the subsurface. The heated probe mobilizes contaminants which are transported to the surface by a carrier gas where different detectors can be used to obtain relative concentrations of volatile contaminants (Dietrich and Leven, 2009). Common detectors used are: Flame ionization detector (FID), PID and halogen specific detector (XSD) or electron capture detector (ECD). The combination of detectors gives a respond to the presence of organic contaminants such as hydrocarbons (FID) and chlorinated solvents (XSD or ECD).

MIP combined with field GC measurements was used as *in situ* screening tool for investigation of the vertical distribution of contaminants and the distribution between mother and degradation products at Vadsbyvej and Gl. Kongevej (Damgaard et al., III; Damgaard et al., IV). The results showed that since the XSD and ECD were most sensitive to the higher chlorinated compounds zones where degradation products dominated were not always identified (Figure 6 A, 4.5-5.5 m.b.s.).

Comparing the degree of dechlorination (DOD, calculation of DOD is described in Damgaard et al., III) from the field GC measurements and DOD calculated from the measured concentrations in the intact core samples the overall level of DOD obtained with the field GC correspond to the DOD in the intact core (Figure 6, B and D). As the discretisation with the field GC was lower minor fluctuations in the matrix were not observed which might result in overrating degradation zones if based on MIP alone. However, the overall results were similar which show that semi quantitative methods with compound specific differentiation, such as MIP combined with field GC, can be used to evaluate degradation in the clay till matrix *in situ*. It is though necessary to take discretisation into account. To overcome this problem it would be useful with continuous analysis of the chlorinated compounds instead of a measurement for every 30 cm. However, method development is needed to obtain this goal.

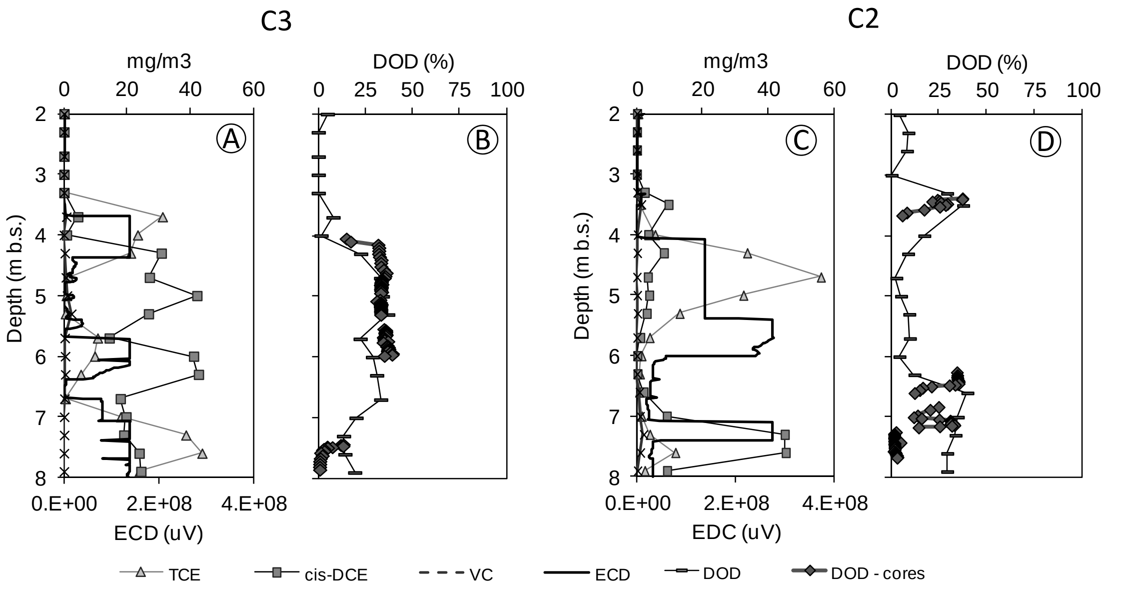

Figure 6: ECD oscillation from a MIP measurement at C3 and C2 at Gl. Kongevej illustrated with the measured distribution of TCE, cis-DCE and VC measured on a field GC (A and C). The calculated DOD from field GC measurements and intact cores is illustrated in B and D.

4.3 MOLECULAR MICROBIAL TOOLS FOR ANALYSIS OF SPECIFIC DEGRADERS

Numerous of microbial/molecular tools that can be used to evaluate degradation processes in contaminated aquifers have emerged (reviewed by Weiss and Cozzarelli, 2008). For the evaluation of degradation of chlorinated ethenes molecular microbial methods have been used to detect and quantify 16S rRNA genes (such as *Dhb*, *Dhc*) and functional genes (reductase genes *tceA*, *vcrA* and *bvcA*)(Cupples, 2008). The functional genes encode for different steps in the reductive dechlorination (described in section 2.3.1) and can therefore be used as indicators for the different degradation steps. It has been suggested in several studies that *vcrA* and *bvcA* are better biomarker for dechlorination as they are not directly correlated to the variation in *Dhc* (Lee et al., 2008; Scheutz et al., 2008; van der Zaan et al., 2010).

Dhc and the presence of functional genes have been detected and quantified at numerous of studies in high permeability aquifers (e.g., Courbet et al., 2011; Fennell et al., 2001; Hunkeler et al., 2011; Lu et al., 2006; Scheutz et al., 2008;

van der Zaan et al., 2010). *Dhc* and functional genes are also able to migrate into low permeability sediment despite the small pore sizes (Damgaard et al., III; Damgaard et al., IV; Manoli et al., 2012; Scheutz et al., 2010; Takeuchi et al., 2011). In a study by Lu et al. (2012) the typical micropore size in Danish clay tills ranged from 1-10 μm and accounted for approximately 30-50% of total porosity. As the size of *Dhc* is smaller (0.3-1μm, Lu et al., 2012) the study supports the possibility of *Dhc* to migrate into the clay till matrix.

Zaa et al. (2010a) found a low and uneven distribution of *Dhc* in aquifer core samples whereas the *vcrA* gene was more uniformly distributed. The results presented in Damgaard et al. (III) and Damgaard et al. (IV) show similar uneven distribution of *Dhb* and *Dhc* as these were detected and not detected in samples with a few cm distance. However, this could also be due to the heterogeneity of the sediment sample.

The activity of the functional genes *vcrA* and *bvcA* can be investigated by mRNA analysis as mRNA is transcribed from the DNA when the cell needs the certain enzyme. Analysis of mRNA has been used to investigate the activity of *Dhc* in microcosm experiments (e.g. Johnson et al., 2005; Lee et al., 2006). The activity of *vcrA* and *bvcA* in permeable aquifers have been reported during natural attenuation (activity (mRNA/DNA) of total *vcrA* and *bvcA* was 6.5-190, Courbet et al., 2011) and after bioremediation (activity of *vcrA* was 1-10 and *bvcA* 0.1-8, Lee et al., 2008). Damgaard et al. (IV) found a higher activity of *vcrA* in the source compared to the plume area after 4 years of ERD in clay till (Gl. Kongevej, Figure 7). The conditions were more reduced and donor was still present, which was not the case in the plume, and a higher activity would therefore also be expected. In contrast the activity was higher at Vadsbyvej but degree of dechlorination was lower. This suggests that more knowledge of the link between the activity, contaminant concentrations, donor availability and redox conditions are needed to directly use activity as a measure for the development of degradation.

mRNA was also sought detected in sediment samples sediment samples from Vadsbyvej and Gl. Kongevej to obtain knowledge about the activity in the clay till matrix. However, mRNA was not detected in any of the sediment samples though the *vcrA* and *bvcA* genes were detected by DNA analysis using standard kit (procedure described in Damgaard et al., IV). Potentially, an analytical limitation could be related to sediment analysis compared to water analysis. The groundwater samples integrate microbial presence and activity over depth (depending on the screen length) whereas sediment samples only represent few g of sediment. Limiting factors in the analysis of sediment can be that mRNA is labile with short half lives (Selinger et al., 2003) and strongly sorbs to the sediment (Chamier et al., 1993).

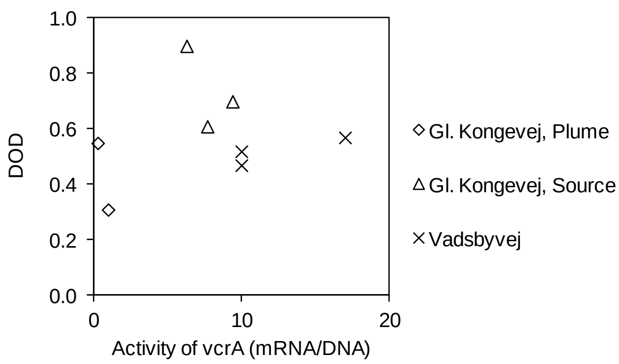

Figure 7: Activities of *Dhc* with the *vcrA* gene at Vadsbyvej and Gl. Kongevej in the source and plume, respectively. Data from Damgaard et al. III and IV.

4.4 CSIA

Carbon in organic compounds consists of two stable carbon isotopes ^{12}C and ^{13}C, of which ^{12}C constitutes the largest fraction. The ratio of the light (^{12}C) and heavy (^{13}C) carbon isotope changes during different processes in the subsurface and CSIA can therefore be used to document and differentiate between these processes. For chlorinated ethenes and ethanes the ratio has been found to change during/after biodegradation (e.g. Fletcher et al., 2011; Hunkeler et al., 2008; Lollar et al., 2010) and abiotic degradation (e.g Broholm et al., 2011; VanStone et al., 2007). More non destructive processes such as dissolution, dispersion and sorption have been found negligible (review by Bombach et al., 2010). However, dissolution and desorption might minimize the observed fractionation by degradation (Chambon et al., V; Blessing et al., 2009; Hunkeler et al., 2008). Diffusion has been found to influence on the isotope ratio in air (Bouchard et al., 2008) and water (Bourg and Sposito, 2008). In the modeling study by Chambon et al. (V) it was shown that with respect to the isotope fractionation in clay till natural degradation processes dominate over diffusion processes.

CSIA have extensively been used to asses/document biodegradation of chlorinated ethenes in high permeability aquifers (e.g. Hunkeler et al., 1999; Hunkeler et al., 2011; Lollar et al., 2001; US EPA, 2008). In the present study CSIA was applied to groundwater samples, but also to sediment samples from the clay till matrix. Analysis of sediment samples had not previously been reported. The procedure for analysis of sediment samples is described in Damgaard et al. (III). However, some method development is still needed as it was not possible to detect $\delta^{13}C$ of the chlorinated ethenes in all samples analyzed. The reason for the lack of detection is currently unresolved.

5 FIELD INVESTIGATION OF ERD IN CLAY TILL

In situ ERD by biostimulation and bioaugmentation has been applied at several low permeability clay till sites in Denmark during the past years; Rugårdsvej (pilot scale), Sortebrovej, Vesterbrogade and Gl. Kongevej (Figure 8). The performance of ERD in clay till highly depends on the progression of degradation in the clay till matrix (Chambon et al., 2010; Lemming et al., 2010). In the present study the development of degradation was investigated at a site which has been undergoing natural attenuation for decades (Vadsbyvej, Damgaard et al., III) and a site where full scale ERD has been implemented (Gl. Kongevej, Damgaard et al., IV). The development of degradation in the clay till matrix has also been investigated by Scheutz et al. (2010) and Manoli et al. (2012) at Rugårdsvej and Sortebrovej, respectively. Details of the different sites where degradation of chlorinated ethenes and ethanes (only Vadsbyvej) has been investigated in clay till are given in Table 5.

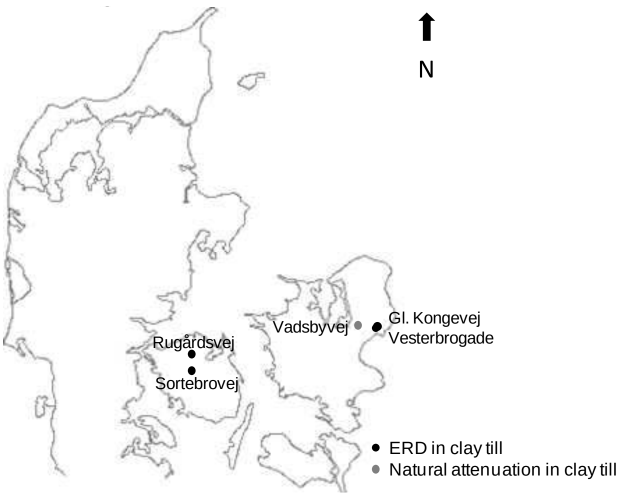

Figure 8: Overview of Danish sites where ERD have been applied in pilot (Rugårdsvej) or full scale (Sortebrovej, Gl. Kongevej and Vesterbrogade). At Vadsbyvej natural attenuation of chlorinated ethenes and ethenes has potentially been taking place for decades.

Based on the knowledge obtained through the present PhD study and results reported by Manoli et al. (2012), the following chapter focuses on how electron donor and bacteria have distributed in the clay till after injection by direct push delivery and gravitational injection. Subsequently, the development of degradation of chlorinated ethenes in high permeability features (such as sand lenses, sand stringers and fractures) and in the clay till matrix will be discussed both during ERD in clay till (Damgaard et al., IV; Manoli et al., 2012) and during natural attenuation (Damgaard et al., III). This will lead to an evaluation of ERD in clay till.

Table 1: Overview of sites where degradation of chlorinated ethenes (and ethanes) has been investigated in clay till.

Site	**Rugårdsvej**	**Sortebrovej**	**Gl. Kongevej**	**Vadsbyvej**
Reference	Jørgensen et al., 2007 Scheutz et al., 2010	Manoli et al., 2012 Region of Southern Denmark, 2011 a and b	Damgaard et al., IV Orbicon, 2011	Christiansen et al., I Christiansen et al., II Damgaard et al. III Christiansen et al., 2008
Scale	Pilot test ERD	Full scale ERD	Full scale ERD	Natural attenuation
Year, initiated	2005	2006	2006	Not remediated by ERD
Geological units and clay till type when known	Unit 1 (~7 m): Basal clay till (type B) Unit 2 (~2 m): Melt water sand Unit 3 (~2 m): Melt water silt and clay Unit 4 (~1 m): Melt water sand Unit 5 (~10 m): Clay till (Damgaard et al., 2008)	Unit 1 (~15 m): Basal clay till (upper part could be flow till) (type B) Unit 2 (~1 m): Melt water sand Unit 3 (~10 m): Basal clay till Unit 4 (~1 m): Melt water sand Unit 5 (~20 m): Basal clay till Unit 6 : Melt water sand aquifer (Region of Southern Denmark, 2011a)	Unit 1 (~4 m): Basal clay till (type B) Unit 2 (~5 m): Basal clay till (type B) Unit 3: Chalk aquifer (Damgaard et al., 2008)	Unit 1 (~8 m): Basal clay till (type B) Unit 2 (~7 m): Basal clay till (type A) Unit 3 (~4 m): Sandy/silty till and local sand lenses Unit 4: Bryozo chalk aquifer
Expected transport paths in the clay till	The upper 3-5 m is highly fractured and contains many worm and root holes. Glacial tectonic fractures can potentially be present.	The upper till contained frequent sand lenses and sand stringers. Vertical fracture systems would be expected (Region of Southern Denmark, 2011a) indicating a type B till. The middle clay till contained sand and gravel lenses. Potentially a type A as fractures was not found in the intact cores.	The tills are also referred to as Upper and Lower Copenhagen till The upper 3-5 m was highly fractured. Both tills are expected to be systematically fractured; potentially glacial tectonic fracture types can be expected. However, the lower till was very compact.	The upper 3-5 m is highly fractured and contains many worm and root holes. Glacial fracture types can be expected in the upper till whereas fewer fractures are expected in the lower till.
Primary contaminants	TCE	TCE	TCE	PCE, TCE and 1,1,1-TCA
Injection method (injection depth)	Hydraulic fracturing followed by gravitational injection (~7 m b.s.)	Gravitational injection (10-20 m b.s.)	Direct push injection (2-7 m b.s.) with injection for each 25 cm	Pneumatic fracturing (3-8 m b.s.), Hydraulic fracturing followed by gravitational injection (3-6.5 m b.s.) and direct push injection (0-10 m b.s.)
Contaminated area (m^2)		2100	140 (source), 60 (plume)	
Distance between injection points		8	4	
Injection points		40	56 (source) and 15 (plume)	
Treated depth		10-20	2-7 (source) and 3-5 (plume)	
Electron donor (kgH_2/m^3)		0.08 (source) (Damgaard et al., 2008)	0.19 (source) (Damgaard et al., 2008)	
Bacteria (cells/L)		2.7×10^6 (Damgaard et al., 2008)	3.4×10^6 (Damgaard et al., 2008)	
Investigation of the clay till matrix (days after start up)	150 and 540	672 and 1566	1331	

5.1 Distribution of electron donor and bacteria

The development of degradation in clay till highly depends on the contact between contaminants, electron donor and bacteria (see section 3.4). Consequently, the contact further influences on the remediation timeframes (Lemming et al., 2010). The spreading of electron donor and bacteria in clay till is therefore of great interest.

Different injection techniques have been used/tested in clay till in Denmark (Table 5) (described in section 3.4). The distribution in the subsurface is influenced by the presence of natural features such as fractures, sand lenses and sand stringers, especially at Sortebrovej where gravitational injection was applied. The source zone at Gl. Kongevej was located in a basal clay till between 2-7 m b.s. The basal clay till was of type B suggesting that the clay till had embedded sand stringers and sand lenses and was systematically fractured with glacial tectonic fractures (see section 3.3.1). Direct push delivery was applied thereby, potentially, increasing the number of fractures. At Sortebrovej the source was located in a basal clay till between 13-22 m b.s. The lodgement till contained sand and gravel stringers (Region of Southern Denmark, 2011a). Gravitational injection was applied from 10-20 m b.s. in boreholes screened in 2-3 intervals of 2 meters. The shallower depth and higher number of fractures at Gl. Kongevej compared to Sortebrovej is an advantage for spreading electron donor and bacteria in the clay till.

The spreading of electron donor and bacteria in the clay till after direct push delivery and gravitational injection will be summarized here based on the observations from full scale ERD (Damgaard et al., IV; Manoli et al., 2012; Region of Southern Denmark, 2011b). Finally, the transport from the high permeability features into the clay till matrix will be described.

5.1.1 Direct push Delivery

At Gl. Kongevej electron donor and bacteria were injected by direct push delivery between 2-7 m b.s. for every 0.25 m. In the intact cores, fractures were observed with greater spacing than 0.25 m indicating that donor and bacteria had spread in existing features in the clay till (sand lenses, sand stringer and fractures) above or below the injection interval. The main propagation path for electron donor seems to be soft clay till, sand stringers and sand lenses, as increased concentrations of fermentation product were observed around these features (after 4 years of remediation) (Damgaard et al., IV). However, the development of degradation across 2 m clay till profiles indicates that donor and bacteria was also spread in indiscernible natural or induced fractures corresponding to observations made in tracer injection tests (Christiansen et al., I and II). The spreading was affected by the compaction of the clay till as the spreading in natural/induced fractures was observed in the shallower less compact clay till. The vertical distance between features (fractures, soft clay till, sand lenses and sand stringers) in the clay till varied from 0.02 to 0.61 m in the treated interval.

5.1.2 Gravitational injection

The spreading of electron donor and bacteria after gravitational injection at Sortebrovej (10-20 m b.s.) was restricted to sand stringers and sand lenses with a horizontal extend of 3-5 m (Region of Funen, 2004). No fractures were observed in the intact cores. In the intact cores the vertical distance between the natural sand lenses and sand stringers was found to be down to few centimeters but more commonly ranged from 0.5 to >1m (Manoli et al., 2012).

5.1.3 Transport in the clay till matrix

Similar to the observations of mobile tracers (Christiansen, 2010), VFA from fermentation of the organic substrate diffused into the clay till matrix from sand stringers, sand lenses and fractures at Gl. Kongevej and Sortebrovej. The results from Rugårdsvej reported by Scheutz et al. (2010) indicated that donor constituents were transported by diffusion into the clay till matrix, where fermentation proceeded. It is possible that donor constituents have also been fermented in the clay till matrix at Gl. Kongevej and Sortebrovej.

The analysis of sediment samples from the clay till matrix showed that *Dhc* were able to migrate into the clay till matrix (Damgaard et al., IV; Manoli et al., 2012; Scheutz et al., 2010). Microbes were found at a distance of up to 10-20 cm from observed high permeability features in the intact cores.

5.1.4 Summary

In summary, the results indicate that electron donor and bacteria have been distributed with a closer spacing by direct push delivery than by gravitational injection. This is concluded as electron donor and bacteria were spread in both natural high permeability features and induced fractures during direct push delivery. The vertical distance between high permeability features was 0.02 to 0.61 m and from few cm to >1 m at Gl. Kongevej and Sortebrovej, respectively.

Fermentation products from the donor and possibly the donor itself diffused into the clay till matrix. *Dhc* with the *vcrA* gene also migrated into the clay till matrix.

5.2 Degradation in clay till

Degradation of chlorinated ethenes and ethanes depends on the donor availability for reductive dechlorination and presence of degraders carrying out reductive dechlorination (described in section 2.3). The donor availability, redox conditions and presence of specific degraders in clay till will be described based on ERD at Gl. Kongevej (Damgaard et al., IV) and Sortebrovej (Manoli et al., 2012) and natural attenuation at Vadsbyvej (Damgaard et al., III). In the following sub-section, the development of reductive dechlorination in high permeability features and the clay till matrix will be described.

5.2.1 Donor availability

After biostimulation with Newman Zone™, EOS® and molasses at Rugårdsvej, Sortebrovej and Gl. Kongevej, respectively, increasing concentrations of primary acetate but also propionate were observed in the groundwater, indicating fermentation of donor (Damgaard et al., IV; Manoli et al., 2012; Scheutz et al., 2010)(sum of VFA are presented in Figure 9, A and B for Sortebrovej and Gl. Kongevej, respectively). Newman Zone™ and EOS® contained 4% lactate. As lactate was not detected in the groundwater at Rugårdsvej and Sortebrovej, lactate was expected to have degraded quickly to acetate and hydrogen. In the full scale studies at Sortebrovej and Gl. Kongevej the highest concentrations of acetate and propionate were observed within 1000 days after injection (500 days in the plume area at Gl. Kongevej). Thereafter, the concentrations of VFA decreased (Figure 9, D). Donor was likely still fermenting after 4 years of remediation, as methane was still being produced at both locations (not in the plume at Gl. Kongevej). However, the methane concentrations were decreasing in the source area at Gl. Kongevej.

After 4 years of remediation, acetate and propionate was present in the clay till matrix at Sortebrovej (Region of Southern Denmark, 2011b) and Gl. Kongevej (Damgaard et al., IV). The concentration of VFA was higher close to soft clay till, sand lenses and sand stringers than fractures. The concentration levels in the clay till matrix were higher than the observed concentrations in the high permeability features (Damgaard et al., IV; Manoli et al., 2011). This suggests that the fermentation of acetate and propionate was faster/easier in the high permeability features. The concentration gradient between the clay till matrix and the high permeability features indicates back diffusion of acetate and propionate from the clay till matrix to high permeability features.

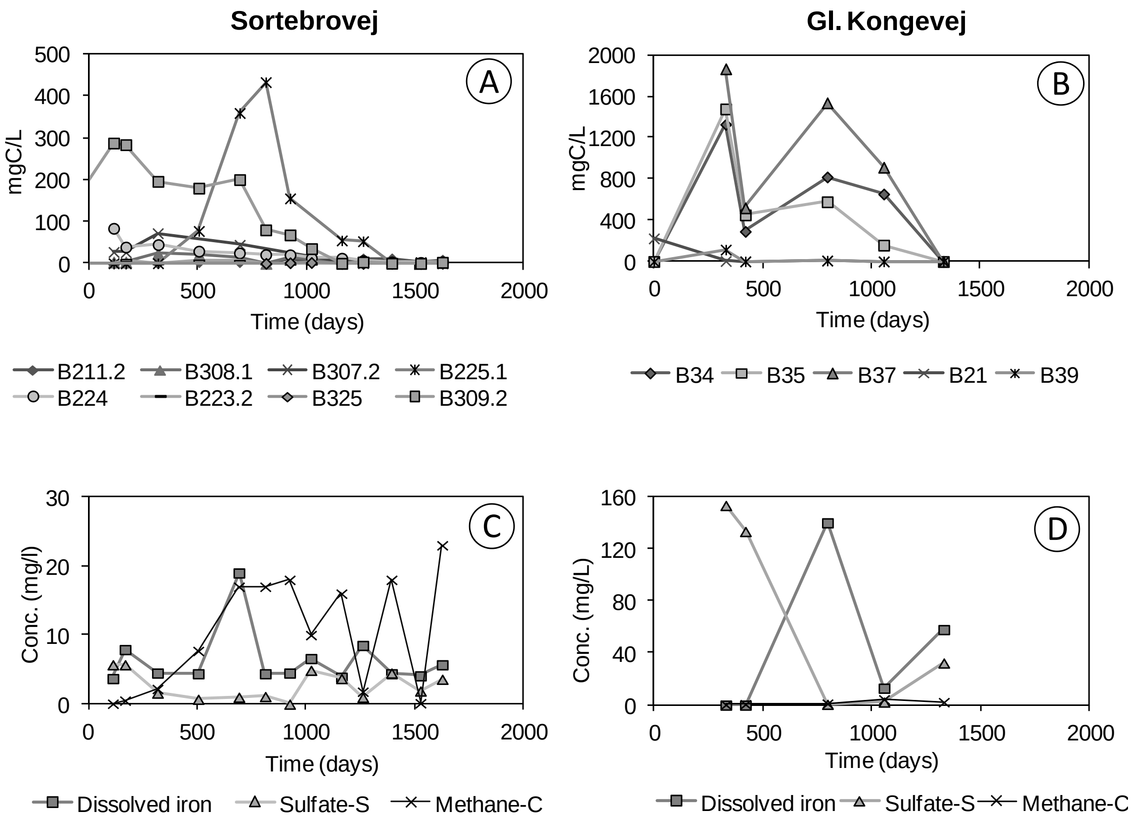

Figure 9: Sum of acetate, propionate, formate and lactate in the groundwater during the monitoring period at Sortebrovej (A) and Gl. Kongevej (B). Below the redox sensitive parameters dissolved iron, sulphate and methane are shown by the representative boreholes for Sortebrovej (C, B224) and Gl. Kongevej (D, B37), respectively. Data from Region of Southern Denmark (2011b) and Damgaard et al. (IV).

Although biostimulation was not applied at Vadsbyvej, high concentrations of mainly propionate but also acetate were measured in both groundwater and the clay till matrix (Damgaard et al., III). The high propionate and acetate levels are likely a result of fermentation of organic contaminants such as hydrocarbons and alcohols, as these were present where high concentrations of propionate and acetate were found. Similar to the observations at Sortebrovej and Gl. Kongevej, the concentrations were lower in the groundwater than in the clay till matrix (Damgaard et al., III).

In summary, donor for reductive dechlorination and respiration of natural electron acceptors was available in the clay till at all sites. However, the decreasing concentrations of acetate and propionate in the groundwater at Gl. Kongevej and Sortebrovej could indicate that donor levels were depleting.

5.2.2 REDOX CONDITIONS

After biostimulation, reduction of natural electron acceptors was observed in the high permeability features thereby creating more reduced conditions (iron reducing to methanogenic, Figure 9, C and D) (Damgaard et al., IV; Manoli et al., 2012). There was a good fit between the effect of the donor and the development of the redox conditions in the groundwater at both Sortebrovej and Gl. Kongevej (Figure 9). When the concentration of acetate and propionate decreased in the groundwater, methane production also decreased and a rebound of iron and sulfate was observed. No or low concentrations of hydrogenesulfide were measured in the groundwater presumably due to precipitation with dissolved iron (Damgaard et al., IV; Region of Southern Denmark, 2011b).

The redox conditions in the clay till matrix were predominantly iron reducing at all investigated locations (Damgaard et al., III; Damgaard et al., IV; Manoli et al., 2012; Scheutz et al., 2010). No nitrate was detected and sulfate was found in all sediment samples. Methane production was restricted to minor sporadically distributed sub-sections.

Iron oxides have been reported as one of the major competing natural electron acceptors for electron donor in the subsurface (Kouznetsova et al., 2010; Zaa et al., 2010; ESTCP, 2004; Heron and Christensen, 1995; Heron et al., 1994). The bioavailability of the iron minerals depends on the chemical structure. The least bioavailable structure is crystalline iron, while amorphous iron is more bioavailable. In the present study, analysis for bioavailable iron was carried out for selected sediment samples at Vadsbyvej. A concentration of 20-57meq/kg bioavailable iron was found. The process of iron reduction has the potential to inhibit/limit the degradation of chlorinated ethenes, as more energy can be obtained by iron reduction than reductive dechlorination (US EPA, 1998). However, a recent study by Wei and Finneran (2011) suggests that iron reduction not only competes with but may also assist reductive dechlorination as both *Dhc* and iron reducing bacteria were enriched concurrently in batch experiments.

In summary, the redox conditions in the groundwater were reduced after addition of electron donor. The conditions were iron reducing to methanogenic. As the donor was depleted the redox conditions became less reduced. The redox conditions in the clay till matrix were predominantly iron reducing with minor sporadically occurring methanogenic area at all locations.

5.2.3 PRESENCE OF *DHB*, *DHC*, *VCRA* AND *BVCA*

Dhc were not present prior to remediation. After bioaugmentation *Dhc* were present in the groundwater at both Sortebrovej and Gl. Kongevej in higher numbers than the injected amount (Figure 10 and Table 5). After the bioaugmentation the number of *Dhc* was slightly increasing at Sortebrovej. At Gl. Kongevej the number of *Dhc* in the source area was relatively stable (B34, B35 and B37) whereas the number of *Dhc* in the plume at Gl. Kongevej was slightly decreasing (B21 and B39, Figure 10, B). The decreasing number of *Dhc* in the plume could be due to lack of donor (Figure 9).

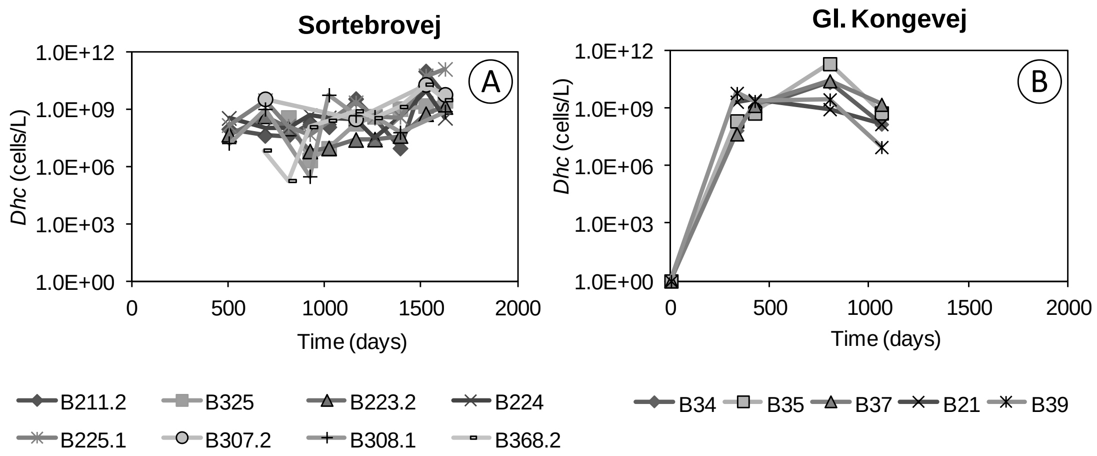

Figure 10: *Dhc* in the groundwater during the monitoring period at Sortebrovej (A) and Gl. Kongevej (B). Borehole B34, B35 and B37 at Gl. Kongevej are located in the source area whereas B21 and B39 are located in the plume. Data from Region of Southern Denmark (2011b) and Damgaard et al. (IV).

After 4 years of remediation at Gl. Kongevej, *Dhc* with the *vcrA* and *bvcA* genes were proven active in all boreholes sampled in the source and plume area as *vcrA* and *bvcA* mRNA were detected (Figure 7). The activity was higher in the source where DOD was also higher (Figure 7). DOD had previously been higher in the plume, indicating that the activity here also has the potential to be higher, but it had decreased due to donor limiting conditions (Damgaard et al., IV).

The presence of degraders in the clay till matrix was not investigated at Sortebrovej and Gl. Kongevej prior to remediation. As they were not present in the groundwater they would though not be expected to be present in the clay till matrix either. After 4 years of remediation, *Dhb* (only Gl. Kongevej), *Dhc* and *Dhc* with the *vcrA* gene were present in the clay till matrix with a vertical distance of up to 12 and 5 cm from high permeability features or fractures at Gl. Kongevej (Figure 13) and Sortebrovej (Region of Southern Denmark, 2011b), respectively. At Rugårdsvej, *Dhc* were observed within short distances (few cm) to an induced sand filled fracture but also at a presumed vertical distance of 22 to 34 cm after 540 days (Scheutz et al., 2010). The presence of vertical fractures and fracture directions around the intact cores are unknown and thereby the potential distance to fractures could be less than the observed distances.

Dhb and *Dhc* were naturally present in some boreholes at Vadsbyvej (10^5-10^6 cells*Dhb*/L and 10^4-10^7 cells*Dhc*/L) (Damgaard et al., III) and *Dhc* with the *vcrA* gene were proven active in 3 out of 4 screens where *Dhc* was found (Figure 7). Even though DOD was lower at Vadsbyvej, the activity was still higher than at Gl. Kongevej (Figure 7). In the clay till matrix, *Dhb* were more widespread than *Dhc*, which were only found in few samples from one borehole (Damgaard et al., IV)).

At Vadsbyvej and Gl. Kongevej the presence of *Dhc* with the VC reductase genes *vcrA* and *bvcA* was documented in high permeability features where production of ethene was also observed (Damgaard et al., III; Damgaard et al., IV). This is consistent with observations in high permeability aquifers (Hendrickson et al., 2002; Lu et al., 2006; Scheutz et al., 2008). In most of the sediment samples from Gl. Kongevej, ethene was also present where *Dhc* and the *vcrA* gene were found. However, at Vadsbyvej, *Dhc* and the *vcrA* gene were not detected in all zones where the ethene concentration indicated complete degradation in the clay till matrix. The degradation to VC and ethene in these zones were limited, however, as the concentrations were low (Damgaard et al., III). This could indicate that *Dhc* were present, but not in detectable amounts.

5.2.4 REDUCTIVE DECHLORINATION IN GROUNDWATER

Reductive dechlorination of chlorinated ethenes in high permeability features increased after biostimulation and bioaugmentation in the clay till at Sortebrovej and Gl. Kongevej (Damgaard et al., IV; Manoli et al., 2012) (Figure 11, A and B). Before the remediation was started primarily TCE was found in the groundwater whereas the percentage of degradation products cis-DCE, VC and ethene was increasing with time after the injection (Figure 11, A and B). The dechlorination increased as degraders and donor became available and the conditions were more reduced (Figure 9 and 10). At Gl. Kongevej, degradation developed at a slower rate in the source area due to higher contaminant concentrations and desorption of TCE from the sediment compared to the plume with lower contaminant concentrations (Damgaard et al., IV). At Gl. Kongevej, the reductive dechlorination was found to stagnate (when DOD was 0.6 in the source) or decrease (DOD decreased from 0.75 to 0.5 in the plume) as the donor was depleted and the redox conditions became less reduced (Damgaard et al., IV). The dechlorination at Sortebrovej stagnated with a DOD between 0.5 to 0.9 after 2 years of remediation (Region of Southern Denmark, 2011b). The stagnating dechlorination could indicate back diffusion of TCE and cis-DCE from the clay till matrix with continuing dechlorination in the high permeability features, whereas the decrease in the plume at Gl. Kongevej could indicate donor limiting conditions.

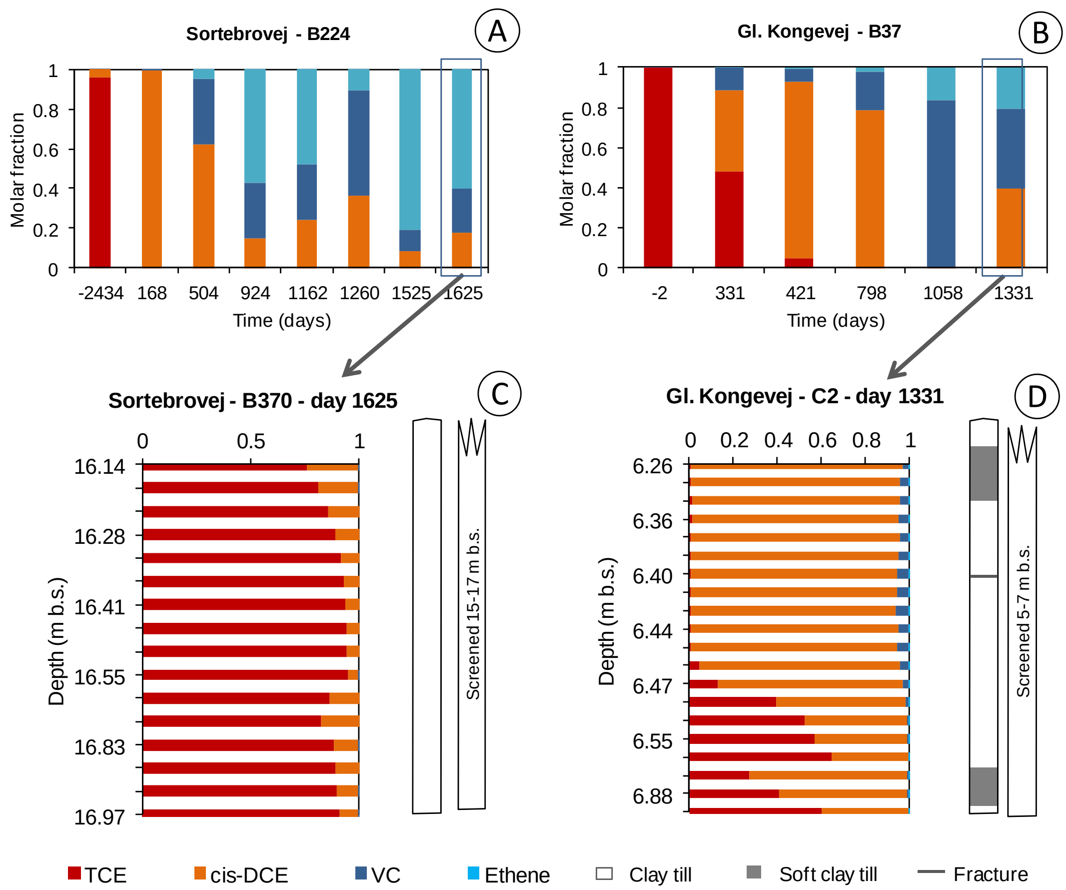

Figure 11: Development of degradation in the groundwater from the start-up of ERD (day 0) in the clay till at Sortebrovej (A) and Gl. Kongevej (B), illustrated as molar fractions in two representative boreholes. The molar fraction of chlorinated ethenes in the clay till matrix at day 1625 and 1331 at Sortebrovej and Gl. Kongevej, respectively, is illustrated in C and D. The geology in and screened depth is shown on the right side of the molar fractions in the clay till cores. Data from Region of Southern Denmark (2011b) and Damgaard et al. (IV).

The investigation of natural attenuation of chlorinated ethenes and ethanes at Vadsbyvej showed that even after decades of attenuation PCE, TCE and 1,1,1-TCA had mainly been degraded to cis-DCE and 1,1-DCA (Damgaard et al., III), even though the redox conditions were favorable for reductive dechlorination, electron donor was available and *Dhc* with the *vcrA* and *bvcA* genes were present and active in the high permeability features.

5.2.5 REDUCTIVE DECHLORINATION IN THE CLAY TILL MATRIX

Investigation of intact core samples from Sortebrovej and Gl. Kongevej showed that the development of reductive dechlorination in the clay till matrix was very heterogeneous (Figure 12). At both sites degradation developed in narrow zones (few cm) around features (sand stringers, sand lenses and soft clay till) in some parts and in others degradation through entire clay till sections developed (up to 1.8 m at Gl. Kongevej)(Damgaard et al., VI; Region of Southern Denmark, 2011b). At Gl. Kongevej degradation was restricted to narrow zones around features where the clay till was very firm and in sections where the clay till was firm degradation developed through entire clay till sections. At Sortebrovej no explanation was found for the more progressed degradation in one profile compared to the other (Region of Southern Denmark, 2011b). However, it could be due to collection of a core next to a vertical fracture (Figure 12, B). In Figure 13 an example is shown for Gl. Kongevej where reductive dechlorination of TCE to cis-DCE was developing in narrow zones (few cm) around features (C2) and through the entire clay till matrix (C3 and C1)(Damgaard et al., IV). Degradation of TCE, cis-DCE and VC in the clay till matrix was documented by enriched isotope fractionations of TCE, cis-DCE and VC (Damgaard et al., IV).

The observations at Gl. Kongevej suggests that a better contact between the contaminants and amendments was obtained by direct push delivery in areas where the clay till was firm compared to areas where the clay till was very firm. Further, the contact obtained by direct push delivery was better than what was obtained by gravitational injection as the vertical distance between features was smaller (see section 5.2). However, direct push delivery at Gl. Kongevej was carried out at shallower depth (2-7 m b.s.), where more natural fractures would be expected. It is also unknown how efficient direct push injection would be at Sortebrovej, as the main contamination was located between 13 and 22 m b.s., where results of direct push have not been reported.

At Gl. Kongevej, *Dhb* was the dominating species where TCE was present (Figure 13, C2), whereas *Dhc* and the VC reductase gene *vcrA* were found in higher numbers where TCE was completely degraded (Figure 13, C3 and C1). In part of the sub-section with complete degradation of TCE to cis-DCE, concentrations of VC and ethene indicate beginning complete degradation to ethene. This was supported by the presence of *Dhc* with the *vcrA* gene (Figure 13, C3). These results suggest that the microbes might be present and start to grow when the conditions are favorable. This could be when TCE is completely degraded as this has been found to inhibit *Dhc* (Yu et al., 2004).

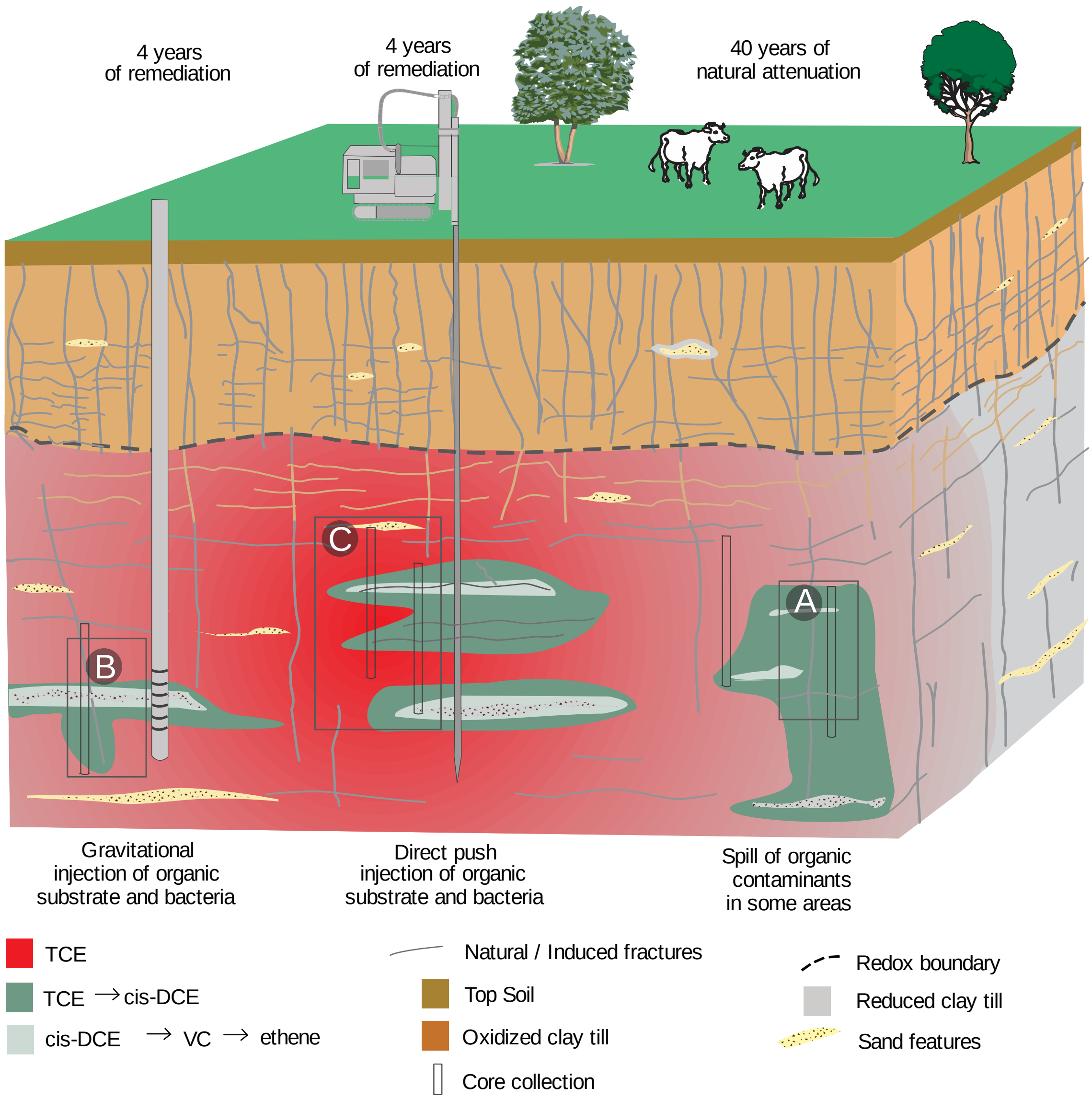

Figure 12: Conceptual illustration of the heterogenic development of degradation in the clay till matrix based on water and core samples. The development of degradation is illustrated after 4 years of ERD using gravitational injection and direct push delivery and after 40 years of natural attenuation at a site where organic contaminants were present as co-contaminants. Further implications related to the representation of samples are illustrated. A: illustrates how a vertical or fracture can be present next to the depth where a core i collected whereby the distance to a feature is overestimated. Similar to A, B illustrates how the development of degradation can be overestimated. C: illustrates how the development of degradation varies within short distances.

The results from the intact cores at Sortebrovej and Gl. Kongevej document that biostimulation and bioaugmentation enhanced reductive dechlorination of chlorinated ethenes in the treated clay till matrix (Damgaard et al., VI; Region of Southern Denmark, 2011b). Minor or no degradation was observed in the untreated areas. The development of degradation in the clay till matrix did not advance as fast as observed in the high permeability features (Figure 11, D). Neither did it represent areas where no features were observed (Figure 11, C). These results show that investigation of the groundwater alone does not illustrate the degradation in clay till, especially not since the degradation in the clay till matrix is very heterogeneous (Damgaard et al., IV; Region of Southern Denmark, 2011b).

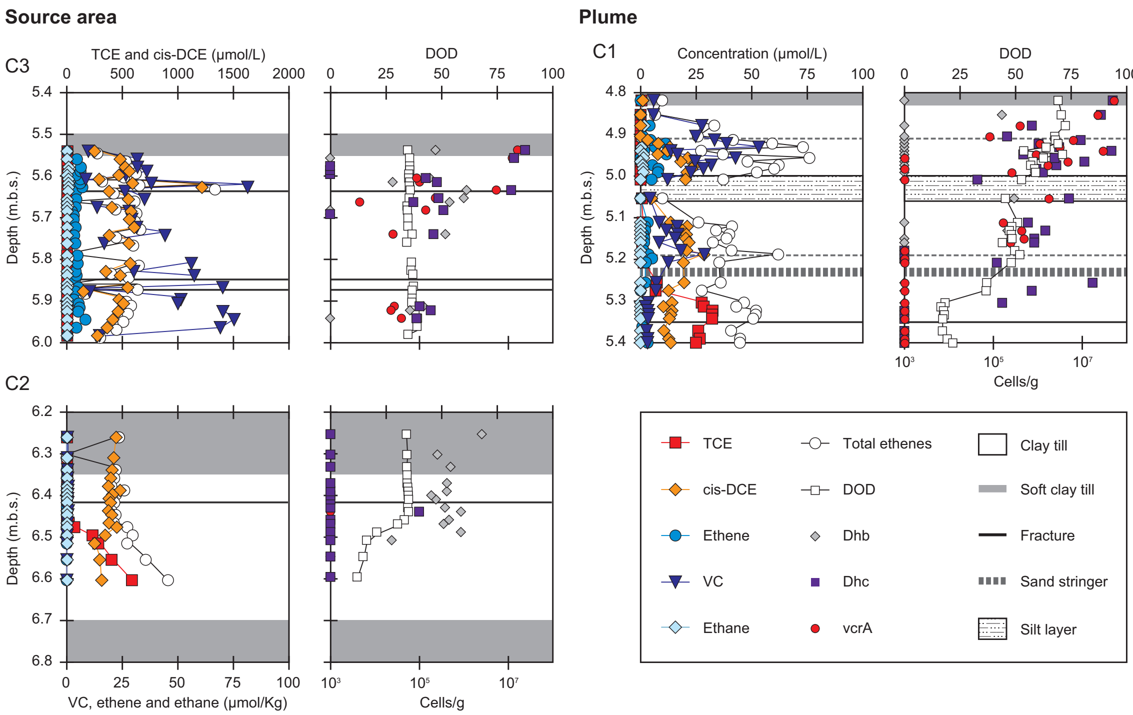

Figure 13: Development of degradation though the entire clay till matrix (C3 and C1) and development of complete degradation in narrow zones around features (C2) illustrated by concentrations of chlorinated ethenes and presence of *Dhb*, *Dhc* and *Dhc* with the *vcrA* gene. Data from Damgaard et al. (IV).

At Vadsbyvej, natural attenuation has potentially been taking place for decades (Damgaard et al., III). The conditions for dechlorination of PCE, TCE and 1,1,1-TCA to cis-DCE and 1,1-DCA were favorable in most of the clay till matrix, as the redox conditions were iron reducing, donor was available and *Dhb* were present. *Dhb* can have served to degrad TCE and 1,1,1-TCA to cis-DCE and 1,1-DCA, respectively (review by Scheutz et al., 2011; review by Middeldorp et al., 1999). Support for this assumption was found as cis-DCE and 1,1-DCA were the dominating compounds in most of the clay till matrix. However, in one profile degradation was limited due to desorption or dissolution of TCE from the matrix. Only minor sub-sections with partial reductive dechlorination to ethene were observed sporadically. *Dhc* were only detected in one sub-section indicating complete degradation to ethene. Degradation of PCE, TCE, cis-DCE and 1,1,1-TCA was documented by enriched $\delta^{13}C$ values for TCE, cis-DCE and 1,1,1-TCA. Enriched $\delta^{13}C$ for VC was also found which supports some further degradation of VC to ethene. The development of degradation of TCE through entire sub-sections of up to 3 m indicate that there is a potential of degradation to develop more extensively in clay till. However, complete degradation to ethene seems to be limited by the lack of *Dhc* with the *vcrA* gene.

5.2.6 ABIOTIC DEGRADATION IN THE CLAY TILL MATRIX

In the study of natural attenuation at Vadsbyvej, highly enriched $\delta^{13}C$ values for cis-DCE were found in groundwater from one screen and in sediment samples from one selected core sub-section (Damgaard et al., III). In the core sub-section, highly enriched $\delta^{13}C$ values for 1,1,1-TCA were also observed. The $\delta^{13}C$ values were more enriched than what would be expected from biotic reductive dechlorination alone. Batch calculations and modeling were therefore carried out and suggested that both abiotic and biotic degradation occurred (Chambon et al., V). Combined abiotic and biotic degradation of TCE and cis-DCE was also observed in a microcosm study with crushed sandstone by Darlington et al. (2008). The abiotic degradation of TCE and 1,1,1-TCA can likely have proceeded by biotic FeS formed from iron and sulfate reduction. However, the results from the study suggest that more knowledge and awareness of combined abiotic and biotic degradation processes in iron-rich sediments are needed. Further, more research on abiotic enrichment factors is needed, as abiotic enrichment factors are not available for all chlorinated ethenes and ethanes (Chambon et al., V).

Abiotic degradation of chlorinated ethenes and ethanes was only documented by isotope analysis. Another parameter which could be used as an indicator for abiotic degradation is acetylene as suggested by Butler and Hayes (1999), since this is the major product from abiotic transformation (Figure 2). However, acetylene is rapidly transformed further (Liang et al., 2009) and may therefore reflect less abiotic degradation than is actually taking place. Microcosm experiments have also been used to document abiotic degradation (e.g. Kennedy et al., 2006; Ferrey et al., 2004).

5.3 Evaluation of ERD in clay till

Monitoring of ERD in clay till includes process monitoring where donor and bacteria availability is followed and performance monitoring where the development of degradation in the clay till matrix is investigated. In the following section focus will be on performance monitoring methods in the clay till matrix. This will be followed by an evaluation of ERD timeframes in clay till based on the results obtained at Gl. Kongevej (Damgaard et al., IV) and Sortebrovej (Manoli et al., 2012; Region of Southern Denmark, 2011b). Finally, side effects, advantages and disadvantages of ERD in clay till are discussed.

5.3.1 Performance monitoring

Degradation of chlorinated ethenes was found to advance faster in the groundwater compared to the clay till matrix after ERD in clay till (Figure 11). As the groundwater reflects the development of degradation in high permeability features, which were also found to constitute the main propagation conduits for electron donor and bacteria, the most favourable conditions for reductive dechlorination are reflected in the groundwater results. The results show that the monitoring of groundwater alone gives a misleading picture of the remediation efficiency (Figure 11). Monitoring of the development of degradation in the clay till matrix is therefore needed to ensure a realistic evaluation of timeframes of ERD in clay till.

The development of degradation in the clay till matrix was very heterogeneously distributed (Damgaard et al., III; Damgaard et al., IV; Region of Southern Denmark, 2011b). All studies at low permeability sites show that the progression of degradation in the low permeability matrix can be investigated by discrete sub-sampling of intact cores. However, the discretisation and number of parameters analysed make the method expensive and time consuming. Hence, simpler and less costly methods are needed.

The distribution and development of degradation can e.g. be investigated by semi-quantitative measurements combined with compound specific analysis. The comparison of results from MIP combined with field GC and intact core sampling showed a relatively good correlation, suggesting that this could be a viable option (described in section 4.2). However, overrating due to lower discretisation has to be considered. Further, quantitative analysis of chlorinated ethenes and ethanes are still necessary to make mass estimates at a site.

5.3.2 TIMEFRAME OF ERD IN CLAY TILL

The timeframe of remediation via ERD highly depends on the development of degradation in the clay till matrix (Lemming et al., 2010). The investigation of development of degradation in the clay till matrix from discrete sampling of intact cores at Sortebrovej (Manoli et al., 2012) and Gl. Kongevej (Damgaard et al., IV) showed a very heterogeneous development of degradation in the clay till matrix (described in section 5.2.5). However, three types of development of degradation in the clay till matrix were observed (also illustrated in Figure 13):

I. Degradation was developing in the entire clay till matrix
II. Degradation was developing from features such as sand lenses and fractures and into the clay till matrix resulting in narrow reaction zones of few cm in the clay till matrix
III. No degradation was developing

These observations were used to assess the remaining mass and timeframes for the remediation at Gl. Kongevej and Sortebrovej. The numerical model presented by Lemming et al. (2010) was used for Gl. Kongevej whereas the numerical model presented by Manoli et al. (2012) was used to simulate remediation timeframes at Sortebrovej. Simulations at both locations included degradation (same reaction parameters were used) and transport of chlorinated ethenes and no donor limitations. The numerical modeling showed that contaminants would leach to the underlying aquifer for up to 1500 years at both locations if no remediation was applied. The shortest remediation timeframes could be obtained if degradation developed in the entire clay till matrix (Figure 14). Comparing the clean up time for degradation in the entire clay till matrix at Sortebrovej and Gl. Kongevej the timeframe for Sortebrovej is much faster. This is due to differences in the initial TCE concentration at the two sites (~67 mg/kg and 4.5 mg/kg at Gl. Kongevej (Damgaard et al., IV) and Sortebrovej (Region of Southern Denmark, 2011b), respectively). The remediation timeframe are more similar at Sortebrovej and Gl. Kongevej when degradation is restricted to narrow reaction zones around sand stringers and sand lenses. This is due to the larger distance between these features at Sortebrovej compared to Gl. Kongevej.

As degradation was developing heterogeneously in the clay till matrix different timeframes can be expected for different areas in the clay till. This is seen by comparing weighted average of DOD for selected core depths with the modelled results (Figure 14). According to these data the remediation timeframe for areas with most progressed degradation will be more than 50 years at Sortebrovej and 20 years at Gl. Kongevej, whereas areas with degradation restricted to narrow zones will be remediated during more than 50 years. The limited mass removal in some areas is likely due to limited distribution of organic substrate and bacteria whereby also degradation is limited. This suggests that there is still a need for more efficient injection methods where organic substrate and bacteria are distributed more uniformly. E.g. recent laboratory experiments have shown promising results for electrokinetic injection in clay till (Mao et al., 2012).

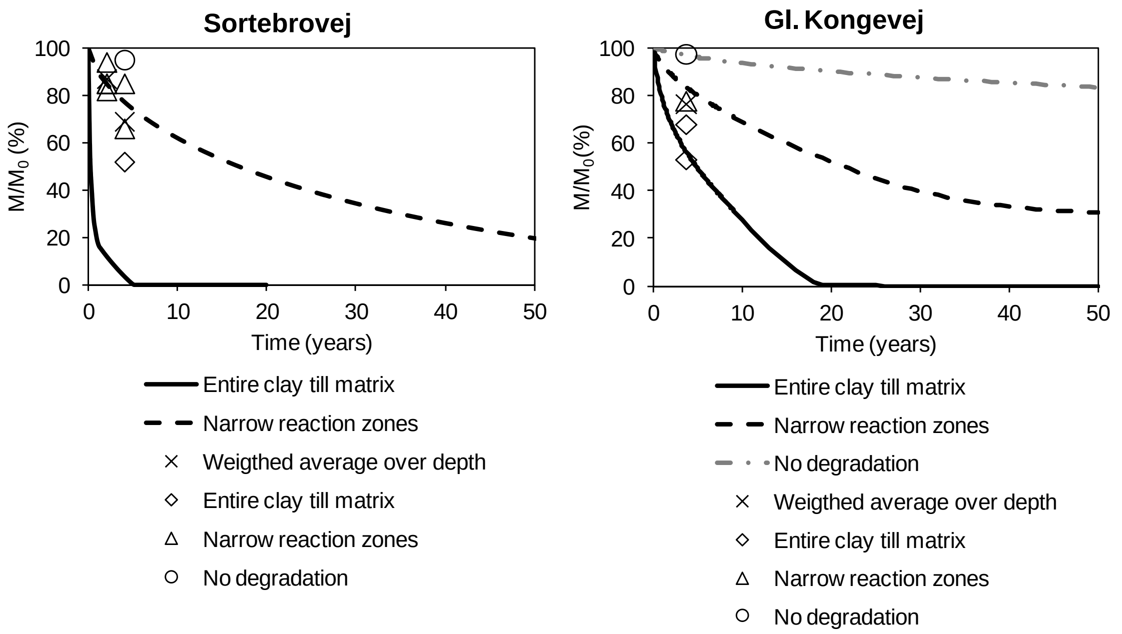

Figure 14: The simulated (continuous lines) and estimated (data points) remaining mass (M/M_0, given as 100% minus DOD, assuming mass conservation) in the clay till matrix at Sortebrovej and Gl. Kongevej. Data from Region of Southern Denmark (2011b) and Damgaard et al. (IV).

5.3.3 SIDE EFFECTS

A potential side effect of ERD in clay till is the production of carcinogenic VC during the degradation process or due to incomplete degradation (Damgaard et al., IV). If complete degradation to ethene is achieved, the side effect is temporary. The side effect takes on a more permanent and, hence, detrimental nature if incomplete degradation is achieved (Damgaard et al., IV). At Gl. Kongevej a cis-DCE and VC plume was formed in the underlying chalk aquifer. According to a modeling scenario of ERD in clay till by Lemming et al. (2010) VC could potentially be the primary compound leaking to the underlying aquifer for about 40 years. Increasing VC concentrations were also observed in the pilot test study at Rugårdsvej by Scheutz et al. (2010). These observations suggest that remediation of a source zone overlying a high permeability layer should include establishment of an active buffer zone in the underlying aquifer to ensure that VC is further degraded to the non-toxic ethene.

Another side effect of ERD is methane production due to anaerobic degradation of substrate. High methane concentrations can pose a risk of explosion if it migrates into an oxygen-rich environment and a spark occurs (Fennell and Gossett, 1998; Fennell and Gossett, 1999; Lee et al., 2004). High methane concentrations were observed at Gl. Kongevej and created the need for establishment of ventilation. High methane concentrations were also observed at Sortebrovej but not near or under buildings.

5.3.4 ADVANTAGES AND DISADVANTAGES OF ERD IN CLAY TILL

Several advantages and disadvantages can be mentioned with regard to ERD in clay till. One advantage of the method is that it is practical in urban areas as injection methods applicable within and under buildings are available (e.g. direct-push delivery). The investigation of progression of reductive dechlorination at Sortebrovej and Gl. Kongevej points out that enhanced injection methods are needed to create the necessary contact between the contaminants and amendments to obtain reasonable remediation timeframes. The average estimated timeframe at Gl. Kongevej was at least 50 years (Damgaard et al., IV) whereas the average remediation time at Sortebrovej was estimated to at least 170 years (Region of Southern Denmark, 2011b). These are very long timeframes considering that much shorter timeframes can be obtained by use of other more aggressive methods (e.g. 3 months by use of In Situ Thermal Desorption (Lemming et al., 2010)). The timeframe of remediation using ERD in clay till therefore appears to be a barrier for the remediation technology.

The results of the investigation in the present study and the study at Sortebrovej show that additional injection will be necessary after 4-6 years (Damgaard et al., IV; Region of Southern Denmark, 2011b; Manoli et al., 2012). This was also suggested by the modeling results from Sortebrovej (Manoli et al., 2012). At both Sortebrovej and Gl. Kongevej some challenges can be expected linked to re-injection. During the monitoring at Sortebrovej a “butter”-like substance was found clogging some screens after injection of organic substrate by gravitational injection. This can complicate reinjection in the boreholes (Region of Funen, 2006). A limitation with the direct push delivery is that the pressure from the re-injection might “blow” into the old injection holes if not securely blocked whereby the donor spreading in the sediment becomes limited. On the other hand re-injection by direct push delivery can be directed at “new” levels that were less successfully treated. Results of re-injection in clay till have not yet been reported/investigated.

Compared to other remediation technologies ERD, is relatively simple to implement. After injection is carried out the remediation is more or less autonomous, i.e. it requires very little maintenance, as only monitoring (both groundwater and clay till matrix) and re-injection every 4-6 years is necessary. The low maintenance and low electricity use of the system was found to be an advantage in a life-cycle study comparing excavation, ERD and In Situ Thermal Desorption (Lemming et al., 2010) in clay till, as remediation by ERD reduced life-cycle impacts remarkably compared to the two other methods.

6 CONCLUSIONS

The aim of the present PhD study has been to investigate the development of reductive dechlorination of chlorinated solvents in clay till to obtain knowledge of degradation processes and to evaluate ERD as a remediation technology in clay till. The following key findings have been made:

- High resolution sub-sampling of clay till cores was necessary to obtain the fine scale heterogeneity and identification of active degradation zones in the clay till matrix. The study of natural attenuation demonstrated that an integrated approach combining chemical analysis, molecular microbial tools and compound specific isotope analysis (CSIA) was required in order to document biotic and abiotic degradation in a clay till matrix.
- Amendment-comparable tracers were distributed in sub-horizontal fractures for every 10 and 25 cm with direct push injection. A horizontal propagation/extend of at least 1 m was obtained between 2.5 and 9 m b.s. Hydraulic fracturing was only successful in 3 m b.s. where a horizontal sand fracture with a diameter of approximately 3.5 m was created. Between 6-9.5 m b.s hydraulic fracturing was not successful as single hydraulic fractures or as multi fractures.
- Natural sand stringers, sand lenses and fractures constituted the primary propagation conduits for electron donor and bacteria after injection by gravitation and direct push delivery. Electron donor and bacteria also spread in induced fractures during direct push delivery. Donor fermentation products diffused and bacteria migrated into the clay till matrix.
- After direct push delivery of electron donor and bacteria in clay till bioactive sections of up to 1.8 m had developed in the clay till matrix, but sections, where degradation was restricted to narrow zones around sand lenses and stringers, were also observed.
- The development of degradation in the high permeability features was more pronounced than in the clay till matrix. Monitoring of the clay till matrix is therefore necessary to obtain a realistic evaluation of the performance of ERD in clay till.
- Remediation timeframes for ERD in clay till were estimated to vary from 20 years in areas where degradation develops in the entire clay till matrix to more than 50 in areas where degradation develops in narrow zones around sand stringers and sand lenses.

7 FUTURE RESEARCH

In this thesis degradation processes of chlorinated solvents in clay till was investigated to evaluate ERD as a remediation technology in clay till. During the investigations it was clear that more knowledge is needed to understand and investigate natural and enhanced processes and to obtain shorter remediation timeframes. Suggestions for future research areas are listed here:

- The study of natural attenuation of chlorinated ethenes and ethanes documented that both biotic and abiotic degradation was taking place in the clay till. More knowledge of the interaction and reaction rates between biotic and abiotic degradation of chlorinated ethenes and ethanes are needed. Further research could also be made on the potential of combining biotic and abiotic degradation. Combining biotic degradation with abiotic degradation the production of toxic degradation products could likely be limited.

- In the present study the activity of *Dhc* was measured through analysis of the mRNA of the functional genes *vcrA* and *bvcA*. More knowledge of the dynamics in the activity over time under different growth conditions is needed to potentially use activity measurements to evaluate development of the dechlorination.

- Laboratory investigations of the degradation of chlorinated ethenes and ethanes in clay till obtaining the discritisation level presented in this thesis are rather extensive and time consuming and are not suitable for commercial use. There is therefore a need for *in situ* screening tools that can be used for investigation of the development of degradation in clay till. To be able to evaluate mass removal combined qualitative and quantitative methods are needed.

- The main propagation path for electron donor and bacteria using direct push delivery and gravitational injection was sand stringers, sand lenses and soft clay till. Consequently, degradation was restricted to these features in some areas which resulted in long timeframes for the remediation. These results suggest that there is still a need for enhanced injection methods or alternative methods to achieve a more uniform distribution of electron donor and bacteria to develop degradation more extensively in the clay till matrix whereby shorter timeframes can be obtained.

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9 PAPERS

I. Christiansen, C. M., Damgaard, I., Broholm, M., Kessler, T., Klint, K.E., Nilsson, B., and Bjerg, P.L., 2010. Comparison of Delivery Methods for Enhanced In Situ Remediation in Clay Till. Ground Water Monitoring & Remediation, 30(4): 107-122.

II. Christiansen, C. M., Damgaard, I., Broholm, M. M., Kessler, T., and Bjerg, P. L., 2012. Direct-Push Delivery of Dye Tracers for Direct Documentation of Solute Distribution in Clay Till. Journal of Environmental Engineering, 138(1): 27-38.

III. Damgaard, I., Bjerg, P. L., Bælum, J., Scheutz, C., Hunkeler, D., Jacobsen, C. S., Tuxen, N., and Broholm, M. M., 2011. Integrated characterization of chlorinated ethenes and ethanes degradation in clay till. Manuscript, Submitted.

IV. Damgaard, I., Bjerg, P. L., Jacobsen, C. S., Tsitonaki, A., Kerrn-Jespersen, H., and Broholm, M. M., 2011. Performance of full scale bioremediation in clay till using enhanced reductive dechlorination. Manuscript. Submitted.

V. Chambon J., Damgaard, I., Broholm, M., Hunkeler, D., Binning, B. and Bjerg, P. L. 2011 Isotope data for dechlorination in clay tills – Use of modeling, Technical note.

The papers are not included in this www-version. The papers can be obtained from the Library at DTU Environment.

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